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# The Rothamsted Memoirs on Agricultural Science

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## CROPS: PLANT GROWTH: PLANT PRODUCTS: ACTION OF MANURES

### CROPS.

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| 1. "The Changing Outlook in Agriculture." E. J. RUSSELL. Presidential Address, Section M, British Association Centenary Meeting, London, pp. 1—23 .. .. . | 1931      |

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| 2. "The Effect of Climatic Conditions on the Growth of Barley." F. G. GREGORY. Ann. Bot. Vol. XL, pp. 1—26 ..  | 1926 |
| 3. "A Physiological Study of Varietal Differences in Plants. I. A Study of the Comparative Yields of Barley Varieties with Different Manurings." F. G. GREGORY and F. CROWTHER (with an Appendix by E. S. BEAVEN). Ann. Bot. Vol. XLII., pp. 757—770 .. .. . | 1928 |
| 4. "Physiological Studies in Plant Nutrition. I. The Effect of Manurial Deficiency on the Respiration and Assimilation Rate in Barley." F. G. GREGORY and F. J. RICHARDS. Ann. Bot. Vol. XLIII., pp. 119—161 .. .. .   | 1929 |
| 5. "Physiological Studies in Plant Nutrition. II. The Effect of Manurial Deficiency upon the Mechanical Strength of Barley Straw." F. R. TUBBS. Ann. Bot. Vol. XLIV., pp. 147—160 .. .. .  | 1930 |
| 6. "Studies of the Physiological Importance of the Mineral Elements in Plants. I. The Relation of Potassium to the Properties and Functions of the Leaf." W. O. JAMES. Ann. Bot. Vol. XLIV., pp. 173—198 .. .. .   | 1930 |
| 7. "Increased Scion Vigour Induced by certain Foreign Root-Stocks." W. A. ROACH. Ann. Bot. Vol. XLIV., pp. 859—864 .. .. .   | 1930 |
| 8. "A Note on the Dichotomous Branching of the Main Stem of the Tomato ( <i>Lycopersicum Esculentum</i> )." J. CALDWELL. Ann. Bot. Vol. XLIV., pp. 495—498 .. .. .   | 1930 |

9. "The Early Development of the Root Nodule of Lucerne (*Medicago Sativa*, L.)." H. G. THORNTON. *Ann. Bot.* Vol. XLIV., pp. 385—392 .. .. . 1930
10. "On the Influence of Soil Temperature on the Germination Interval of Crops." J. O. IRWIN. *Jour. Agric. Sci.* Vol. XXI., pp. 241—250 .. .. . 1931
11. "Studies in Sampling Technique: Cereal Experiments. I. Field Technique." A. R. CLAPHAM. *Jour. Agric. Sci.* Vol. XXI., pp. 366—371 .. .. . 1931
12. "Studies in Sampling Technique: Cereal Experiments. II. A Small-scale Threshing and Winnowing Machine." T. WAKE SIMPSON. *Jour. Agric. Sci.* Vol. XXI., pp. 372—375 1931
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14. "Field Observations on Starch Production in the Leaves of the Potato." E. J. MASKELL. *Ann. Bot.* Vol. XLI., pp. 327—344 .. .. . 1927
15. "The Determination of Cellulose in Straws." S. H. JENKINS. *Biochem. Jour.* Vol. XXIV., pp. 1428—1432 .. .. . 1930
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17. "The Effects of Summer Green Manures on the Ammonia and Nitrate Contents of Soils Cropped for Winter Wheat. An Examination of the Woburn Green Manure Plots." T. J. MIRCHANDANI. *Jour. Agric. Sci.* Vol. XXI., pp. 458—468 .. .. . 1931
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| 34. "The Evolution of Dominance in Certain Polymorphic Species." R. A. FISHER. American Naturalist. Vol. LXIV., pp. 385—406 .. .. . | 1930      |

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During these hard years there was a growing struggle between town and country. The Industrial Revolution, which had been going on for some sixty years, was now producing epoch-making results. Village industries were moving into the towns, thus narrowing the range and activities of village life and taking away many of the skilled craftsmen. The Revolution affected political as well as economic conditions. The towns had no desire to be governed by the landowners, and were seeking adequate representation in Parliament; agitation for the Reform Bill was proceeding at an alarming pace, and might have led to civil war, but, as usual in England, common sense prevailed, and in May 1832 Lord Grey was recalled by the King, the Reform Act was passed, and the government of the country passed out of the hands of landowners into those of the middle classes, whose interests were then largely urban. England ceased to be an agricultural country, and became definitely industrial and commercial. The towns had won.

This new orientation of the national life was the dominating factor in 1832, when our hundred-year survey begins. Henceforward the trend of legislation was to be always in favour of the towns whenever their interests conflicted with those of the countryside. The new powers were exercised at once. The rate-aided wage was abolished in 1834 when the new Poor Law system was set up. Agitation was started for the repeal of the Corn Laws. Farmers thought the end was coming.

There was, however, a hopeful feature. The towns were growing and requiring more food; importations from overseas were not serious; labour was abundant and cheap, and there was more and more tendency for the unemployed to move to the towns, thereby reducing the farmers' rates. The path of prosperity lay open to those who could produce more food.

Science now began to come into the picture. It was introduced in England by Sir Humphry Davy, whose lectures at the Royal Institution from 1802 to 1812 on Agricultural Chemistry had attracted widespread attention. He dealt particularly with manures and soils, and discussed the problem, then much agitating farmers, why some soils were so much more favourable to crop production than others; he also introduced a method of soil analysis which was speedily taken up by chemists. The Bath and West, our oldest agricultural society, founded at Bath in 1777, set up an agricultural laboratory in 1806—the first in this country—and appointed Dr. Archer as unpaid 'Chemical Professor to the Society.' Within a few months, however, death, to quote the Society's records,<sup>2</sup> 'put a period to the exercise of his private virtues and public exertions,' and Cadwallader Boyd was appointed as chemist to analyse soils, lime-stones and other things for their members—the first appointment of this kind I have been able to find. But for all the scientific advocacy of soil analysis one cannot see how the data of these days would help the perplexed farmer. The methods showed the percentages of silica, alumina, lime, magnesia, carbonic acid and 'vegetable fibres and extract,' but no interpretations were possible. It is not surprising that no important results were achieved.

<sup>2</sup> Bath Society's Papers, 1807, vol. ii, pp. xiii and 275.

The second entry of science into agriculture was entirely different, and achieved an astonishing, even dramatic, success. Several quite independent movements led up to it. Farmers were themselves beginning to formulate their problems more distinctly. A definite and precise statement was published by the Royal Agricultural Society on its formation in 1838, setting forth the problems which had been perplexing the leading agriculturists for some time, and insisting on the need to 'inquire after causes.'<sup>a</sup>

The programme was extensive, indeed we have not completed it yet. Although its framers may not have known, the 'inquiry after causes' was already well on the way. The striking feature of the new work was the demonstration that the carbon which formed about half the dry matter of the crop came from the carbon dioxide of the atmosphere, not from humus, as the older philosophers had thought; the agricultural significance was not at first recognised. The question of rotation of crops was also being investigated, and in this the British Association had taken a leading part. At its first meeting at York, in 1831, Prof. Lindley had been asked by the Botanical Committee to present 'an account of the principal questions under discussion in Botanical Science,' and in his report he included this of root excretions: 'the necessity of the rotation of crops,' he said, 'is more dependent upon the soil being poisoned than upon its being exhausted.' Daubeny, Professor of Botany at Oxford, was invited to study the question, and at the 1834 meeting he described his plan. Eighteen different crops were to be grown for a period of ten years on the same ground, some continuously and some in rotation. The crops were to be weighed and analysed, and the effects of continuous growth compared with those of the rotation. This was done; it was the first continuous and systematic plot experiment ever made. These various

<sup>a</sup> *Jour. Roy. Ag. Soc.*, 1840, vol. i, p. li.

The problems were:—

1. *Classification of Soils*.—Chemical methods having achieved no decided success, would geological methods be better? To test the possibilities, a survey of the Weald of Kent and Sussex was proposed; this was afterwards put into the hands of William Topley, the founder of Soil Surveys, whose Memoir is one of the great classics of the subject. (*Jour. Roy. Ag. Soc.*, 1872, vol. viii (2nd series), p. 241; also *Memoirs of the Geological Survey*.)

2. *Permanent Improvement of Soils*.—The most effective method of draining.

3. *Productiveness of Seeds*.—Comparison of the productiveness of different crops and varieties on different soils, including the nutritive and other values of the crops.

4. *Manures*.—Studies of farmyard manure, of town wastes, bones, rapeseed, &c., and 'mineral manures,' lime, chalk, gypsum, marl, saltpetre, peat ashes, salt, &c.

5. *Rotation of Crops*.—The influence, sometimes favourable and in other cases hurtful, which various crops exercise on others by which they are followed and which is now supposed to be occasioned by an excrementitious deposit left by the roots of plants in the soil.

6. *Mechanics of Agriculture*.—Studies of implements and machines.

7. *Management of Grassland*.

8. *Physiology of Agriculture*.—More abstract questions, as for instance, that bone manure is beneficial on certain soils, and inefficient on certain other soils—under this head we should inquire after causes and endeavour to answer the question, what is the constituent element of bone that promotes vegetation on some soils, and how is that element rendered inoperative elsewhere?

9. *Livestock and Veterinary Problems and Diseases of Plants*.—No details are given; too little was known about any of them.

investigations into causes did not directly influence agriculture, but they provided the basis on which further development soon came. In 1837 Liebig had attended the Liverpool meeting of the Association, and he urged upon British men of science to study organic chemistry, 'which, when taken in conjunction with the researches of physiology, both animal and vegetable, which have been so successfully prosecuted in this country, may be expected to afford us the most important and novel conclusions respecting the functions of organisation.' The very shrewd promoters of the meeting replied by asking him to prepare a report on the state of Organic Chemistry and Organic Analyses. This never came; instead, in 1840, he published a volume, 'Chemistry in its Application to Agriculture and Physiology,' stating in the introduction that it was a report presented to the British Association, though it is not mentioned in the proceedings of any of the meetings. Without exaggeration this can be described as the most important publication in the whole history of agricultural science. It brought together the results of the plant physiologists and deduced from them the principles underlying the nutrition of plants, emphasising the fundamental importance of the ash constituents, phosphates and potassium, magnesium and calcium compounds, which no one had previously noticed. Prior to that farmers had been told to supply humus as the source of carbon. Liebig pointed out that carbon was one of the things farmers need not supply, as it was present in unlimited quantities in the air; nitrogen also, like carbon, came from the air, and need not be supplied. On the other hand, the ash constituents came from the soil, and might easily be lacking; these, therefore, must be supplied. The proper way of manuring was to provide ash constituents, not organic matter. The new ideas were exceedingly simple; agriculture suddenly became a branch of chemistry backed by the great Liebig himself. The feeding of crops became almost a matter of arithmetic; the ash of a crop contains so much of a certain element, therefore so much must be present in the soil or added in the manure. Men of science rose to the situation. Murchison, President of the Association in 1846, urged agricultural members to make use of the Association for the solution of their problems. 'And if, above all, they wish us to solve their doubts respecting the qualities of soils, or the effects of various manures upon them, our chemists are at hand.' We are grateful to our distinguished President for making no such promise on our behalf at this Meeting. Meanwhile, a much younger man was beginning his work. John Bennet Lawes, the owner of Rothamsted, had studied at Oxford from 1832 to 1834, attending the lectures of Prof. Daubeny and seeing his continuous plot experiments. On his return to Rothamsted he began pot experiments with various plants, and soon found that growth was improved by sulphate of ammonia, a waste product from gas works. This was not a new discovery, but it was not widely known. Further, he tried another waste product, animal charcoal (which contains much calcium phosphate), and found that it too was effective, especially after treatment with sulphuric acid when the soluble phosphate, then called superphosphate of lime, was produced. A neighbouring landowner put to him the Agricultural Society's problem: Why are bones effective on some soils and not on others? He showed that treatment with sulphuric acid was all that was necessary to make them

generally useful. Further, he showed that mineral calcium phosphate gave the same product, and so could be converted into a valuable manure. All this was done before Liebig's report of 1840 appeared; the work was so novel that Lawes was able to take out a patent for it and so to found the artificial fertiliser industry. He did not at once do this, being dissuaded by his friends—in 1838 a gentleman and a landowner did not embark in trade, least of all in the manure trade—he waited till 1842. He set up a factory at Deptford Creek, though I am unable to find the source from whence he obtained his phosphates in the early years, nor indeed can I recover much information about those years; fortunately there is some record, for in 1851 he took proceedings against various persons for infringing his patents, and the papers preserved at Rothamsted tell us much about the history of the discovery.

Simultaneously with all this, however, the field experiments at Rothamsted were developed, and of these the records are very full. They arose out of the pot experiments, but were quickly expanded to controvert Liebig. Lawes recognised that he could not look after both these and the factory, and in June 1843 he brought in Gilbert to have charge of them, giving him as laboratory the barn in which the chemical work had hitherto been done. Lawes, and especially Gilbert, had all the Victorians' love of controversy. They did not attempt to rehabilitate the old humus theory, nor did they dispute the necessity for potash and phosphates; they showed, however, that these so-called mineral manures were not sufficient, nitrogen must also be given; Liebig had denied this. Secondly, they showed that the composition of the plant afforded no guidance as to its manurial requirements. Turnips contained but little phosphate and much potash, yet they responded to phosphatic far more than to potassic fertiliser. Lawes and Gilbert remained faithful all their days to their first love, nitrogen; and both at Rothamsted and many years later at Woburn, the whole scheme of field experiments revolved round this need for supplying nitrogenous fertiliser. The fame of Rothamsted, however, grew up on the three field experiments; on Broadbalk wheat, the most important crop of the time, showing on the untreated land the 20 bushels per acre familiar to the farmers of the 40's, and on the plots treated with the new artificial fertilisers, especially with sulphate of ammonia, the unusually large yields of 35, 40 or even 50 bushels; the Barnfield, where Lawes' superphosphate gave remarkable increases in yield of turnips, the next most important crop; the increases were at least as good as could be obtained with the best farmyard manure, which then, as now, was scarce; and the adjoining Agdell field showed the great value in the rotation of clover, a fact which was not new, but sufficiently little known to make the demonstration very interesting. Never before had an experimental farm such a striking display of new discoveries; never before had it been possible to show how this wonderful science about which people were talking so much, could do so much for agriculture. The Rothamsted fields were the first effective demonstration grounds, and so well did the farmers of the day appreciate Lawes' work that they not only bought his superphosphate, but after only ten years, in 1853, they subscribed £1,160 to build a laboratory which should take the place of the old barn that had been in use for some fifteen years. This laboratory was the first of its

kind, and it remained in use till 1914. Unfortunately the original barn was pulled down by Lawes, so that we are deprived of what would otherwise have been a wonderful historic memorial.

Had Rothamsted simply been a place for the demonstration of artificial fertilisers, its usefulness would soon have passed. But from the outset it was much more. Like Daubeny's plots at Oxford, to which their general plan seems to owe a good deal, and like the very important farm at Bechelbronn, where Boussingault was carrying out his fundamental researches on agricultural science, the purpose of the work was a search 'after causes,' a search for knowledge. Lawes emphasised this very clearly in his speech in 1855 at the opening of the new laboratory. 'I must explain to you, gentlemen,' he said, 'that the object of these experiments is not exactly to put money into my pocket, but to give you the knowledge by which you may be able to put money into yours, to enable you to judge the properties of all your several crops . . . to give you that knowledge which will enable you to pursue that course which would be most profitable to you.' Throughout the stress is on the gaining of knowledge. This early recognition that the purpose of agricultural experiments is to provide information which farmers can use for themselves accounts for the rapid success achieved.

Armed with this new knowledge and the new fertilisers, British farmers continued to increase their production, and the towns continued to buy still more food. The Repeal of the Corn Laws in 1849,<sup>4</sup> while it lowered corn prices on the whole, did not bring them lower than farmers had known, and improved transport and growing demands made sales much easier.

One of the great obstacles of the day was lack of drainage, but in 1845 Scragg had invented the pipe-making machine, and by 1850 there was sufficient money in the countryside to begin those extensive drainage schemes which did so much for our countryside.

All this time the livestock of the country was steadily improving; the Shorthorn was displacing the Longhorn, other important breeds were defined and their special qualities developed. The standard of farming rose high, prosperity increased, land was brought into cultivation, and if there ever was a golden age for agriculture it was in the 60's and 70's of the nineteenth century. Experts came from many other countries to see and to learn. In 1872 the area of land under arable cultivation in Great Britain was no less than 18.4 million acres, the highest it ever reached. The nation was made as nearly self-supporting as was possible. The system required a considerable demand for wheat at 50s. to 55s. per quarter, and a considerable supply of good agricultural labour at about 10s. to 12s. per week; so long as these conditions were satisfied it could continue successfully and indefinitely.

But at the height of its glory the system collapsed. Two causes operated. Labour was not content with the standard of living implied

<sup>4</sup> The Bill was passed in 1846, but did not become operative till February 1849. Trevelyan states that the chief factor was the potato blight in Ireland, which had destroyed the potato crop on which the peasants fed and made cheap wheat vitally necessary. He records Wellington's comment: 'Rotten potatoes have done it they put Peel in this d— fright.'

in a weekly wage of 10s. to 12s. and a 55s. price of wheat, and Joseph Arch started his Union in 1872. Even more important, transport was developing and the new countries were opening up.

The fall began in 1874. Wheat had been 55s. 9d. per quarter on the average for the year. In 1875 it was down to 45s. 2d., a price at which many farmers could hardly grow it, in spite of the low wages. The United States was sending wheat here in quantity and greatly underselling our farmers. Prices in '76 and '78 were hardly any better (though '77 had been), and then in '79 came a terribly wet year, the worst in the century, when wheat all over the country was badly lodged and badly harvested. Farmers' resources had been depleted by the low prices, and now came low yields and a very expensive harvest. In the old days the price would have risen and righted matters, but now importations increased so much that prices fell below 44s. Many farmers were ruined; some hung on hoping for better times, which, however, never came. Another Royal Commission was appointed, and pronounced the distress to be of 'unprecedented severity.' But worse was to come. More and more wheat came from the United States at still lower prices, till in 1894 and '95, through a financial crisis in the Western States, wheat fell to 23s. per quarter as the average for the year, while many farmers had to sell for much less. These very low prices did not benefit the townspeople, and they ruined the countryman, causing terrible distress among labourers and farmers, and shattering completely the wonderful system of agriculture that had taken 100 years to build up. Lawes had to confess that science could do nothing to help; it had increased yields per acre and could do so again, but the trouble was too deep-seated to be cured by higher farming.

How had all this come about? For 200 years American farmers and English farmers had never seriously competed, and now all of a sudden the competition became terribly severe. But there had been this difference between American and British farming. Over there man-power had never been abundant, and from the outset American and Canadian engineers had invented machines to do the work with less labour; they did not, like the British engineers, aim at doing it better or at increasing output per acre; their aim was to increase output per man, and in the struggle between the two the higher output per man had won. These developments had been proceeding for many years, and had been much helped by the admirable system of agricultural education that had grown up in the States. So great was American faith in education that even in 1862, during the anxious days of the Civil War, Justin Morrill had been able to get the Morrill Act passed and signed by Abraham Lincoln, establishing in each State a College of Agriculture. The scope of these colleges was widened in 1887 when another great Act, the Hatch Act, provided federal funds for setting up agricultural experiment stations at each of them; further funds were provided by a supplementary Act in 1890. The American farmer of 1894 was therefore well provided with information. He suddenly became an effective force in the world because the chain of transport arrangements from the prairies to the British ports was then completed. British farmers tried in several ways to meet the situation. Some, like Mecchi of Essex, struggled manfully with the

old system, working it more intensively, but they only failed the worse, as Lawes had told them they would. Instinctively most farmers turned to livestock and laid the land down to grass, but as their capital was exhausted they were unable to do it well; nevertheless much of it by good management came off satisfactorily. Many arable farmers went bankrupt and simply gave up the struggle; many Essex farms became almost derelict. They were taken up by young Scots farmers, attracted by the irresistible lure of getting something for almost nothing. They knew and cared nothing about wheat growing, but they were very competent dairy farmers and potato growers, and by dint of hard work and simple living they succeeded in creating a new agriculture that made the farms solvent once more. Gradually it became recognised that specialisation offered the best way out of the farmers' troubles. Now that transport was so efficient, it was no longer necessary for each country or district to aim at being self-sufficing; instead, each region could confine itself to what it could best produce, and import the rest of its requirements from elsewhere. Specialisation allowed of much more efficient production per man, of the introduction and the fullest utilisation of improved methods, and it required that the farmers should be intelligent, mentally alert, fully cognisant of the properties and peculiarities of the crops or animals they were handling, and organised for successful buying and selling.

Fortunately, just at this time agricultural education was spreading in England. There had been since the middle of the eighteenth century spasmodic efforts at agricultural education at the older universities, Edinburgh having the credit for the most sustained teaching; and in 1842 the Agricultural College at Cirencester was founded, which had a great influence in training landowners and land agents. But there had been nothing to reach the farmer; the great link between science and practice had been the Royal Agricultural Society, with its wonderful experts, Augustus Voelcker, Miss Ormerod, and others. A beginning was made in 1888 when the Departmental Committee, presided over by Sir Richard Paget, reported in favour of State-aid for local centres of agricultural education. In 1889 the Board of Agriculture was founded, and from the outset it adopted the policy of establishing agricultural colleges or departments of universities. The great event, however, was the ear-marking for technical (including agricultural) education in 1890 of the tax on whisky imposed in the first instance for the suppression of licences, but not so used. This so-called 'whisky money' provided the funds out of which the colleges and farm institutes were set up, beginning with one only, Bangor, in 1889, and ending with eighteen in 1900; more have been added since. The movement spread into the village school; for twenty years it had been a common and legitimate cause of complaint in the countryside that rural education had nothing in common with rural life, that it fitted children only for clerical occupations, and was of little or no help to the future farm worker. The Board of Education appointed a special inspectorate to put this matter right; school gardens were set up and courses designed to help the teacher draw on the countryside for educational material. The purpose was not to make farm labourers, but to develop the power of observation, of recording, of thinking, to show the child something of the infinite wonder and glory of the English countryside,

and to impart a background of knowledge that would enrich its life whether it remained in the country or went to the town.

The pioneers of those days—Middleton, Hall, Wood, Gilchrist, Somerville, Percival, F. B. Smith—to name only a few, had a strenuous uphill task. There was teaching in the college to be done, field experiments to supervise, lectures to farmers in those pre-motor days when there were only open traps and long dreary waits for slow trains; often no chance of getting a decent meal, and, what was worse, sometimes an unsympathetic audience hoping that the local funny man sitting in the back row would be able to score off the unfortunate lecturer. People would write to the newspapers protesting against the idea that a college could possibly teach farmers anything of value. News of this got back to the universities and gave agricultural science a rather bad name. But the pioneers kept on with their struggle, and, inspired by the faith that was in them, they carried agricultural education through the length and breadth of the countryside; their teaching has become part of the light by which we now walk.

Then came the system of County Agricultural Organisers. These now play so great a part in British agriculture that one is apt to forget that they began only about 1900<sup>5</sup>; with them have grown up the farm institutes, and now there are springing up everywhere discussion societies where farmers meet to discuss technical and other matters of importance. At first no provision was made for research; then it was realised that agricultural education could not be carried on without research; one could not go on repeating the same lectures year after year without testing the statements and seeking new knowledge. Research on any important scale became possible only after 1909, when the Development Fund of £2,000,000 was set up at the instance of Mr. Lloyd George for a variety of purposes, including research. The Development Commissioners at the outset adopted the wise policy of allocating the several sections of agricultural science to existing institutions, making grants on an adequate basis, and so ensuring a widespread interest and, perhaps more important, a widespread net to capture young and capable research workers. Crop production (soil, plant nutrition and plant pathology) was placed at Rothamsted, animal nutrition at Cambridge and the Rowett Institute, plant genetics at Cambridge and Aberystwyth, animal genetics at Edinburgh, agricultural botany at Cambridge, dairy research at Reading, fruit at Long Ashton and East Malling, economics and engineering at Oxford, horticulture and low temperature research at Cambridge, veterinary research at Cambridge and Weybridge, helminthology at the London School of Tropical Medicine, glasshouse horticulture at Cheshunt. The scheme is worked through the Ministry of Agriculture, and it is one of the best instances of successful combination of Government supervision of finance with adequate freedom of action for the research worker. The general result of all these activities has been that farmers have learned to cheapen production, to seek profitable outlets for their industry, to use

<sup>5</sup> The present widespread system was set up only in January 1919, when the Board of Agriculture, as it then was, circulated to the counties proposals for a comprehensive system of agricultural education, offering to pay 80 per cent. of the organiser's salary and 66½ per cent. of all other approved expenditure.



machinery and any other aids to production. Results soon appeared. When Hall, in the years 1910-1912, made his classical pilgrimage of British farming, he records as his general impression that 'the industry is at present sound and prosperous. . . Rents have definitely risen with the demand for land that cannot be satisfied, and in all parts of the country men are obtaining very large returns indeed on the capital they embarked in the business.' This was less than twenty years after the deep depression of the early 'nineties!

Then came the war. For the English countryside (so far as any men were left), for the overseas Empire and the United States, it was a time of feverish activity to raise more food to sell to the Allies. Prices were fixed in England, so that money never abounded in the countryside as it had done in Napoleonic times. In spite of the sadness of the war years the farmers of Great Britain put up a wonderful fight to produce food. The history of the time has been written by Middleton.<sup>6</sup> After the war came three years of high prices; in 1920 wheat averaged 80s. 10d. per quarter, the highest since 1818. Then just as suddenly there came the slump; by 1922 wheat was down to 47s. 10d. The high prices had done farmers very little good, and in the end they lost all that they had gained. Many landowners proceeded to sell their estates. The high price of produce induced many to bid for the land, and the sitting tenant had either to outbid or be dispossessed. Frequently he had to pay more in interest on loans and mortgages than he had paid in rent, and in addition he has also to maintain the buildings, gates and roads which formerly the estate had done; moreover, as a landowner he has incurred the hearty dislike of some of the town dwellers, who now promise him extra taxation. He is therefore in a far worse position than the farmer of 1821 in the slump after the high prices of the Napoleonic wars. But much worse has come. When the first rush of cleaning up after the Great War was over it was realised that the world's power of producing food had grown far in excess of its power of consuming food. The population had increased but the power of food production had increased much more. In consequence, prices of farm produce have fallen far more than costs of labour and of other commodities. British farmers have turned, as in the 1890's, to livestock, raising lamb, young pigs and milk as far as possible on grass with an increasing acreage of lucerne, thanks to the success of Thornton's inoculation method. Those who cannot produce grass cheaply and easily, but who have to depend on arable land, are in a sorry plight, and the difficulty is not confined to this country; arable farmers in all civilised countries are deeply depressed.

This certainly is not the result that was expected; on the contrary, experts had confidently predicted a food shortage. Sir William Crookes, in his presidential address to the Association in 1898, forecasted the probable world requirement of wheat for the next three decades, and showed that the sources and methods then available would continue to suffice only till 1931, when the world would begin to feel the pinch of hunger. It seemed a tragic ending to the magnificent triumphal march of the nineteenth century. Crookes' figures were remarkably

<sup>6</sup> *Food Production in War*: Oxford (Carnegie Endowment).

accurate, and there can be no doubt that, had science and practice stood still since 1898, we should now be facing the horrors of world starvation. But they have not stood still, and the present position of farm prices is a measure of their advancement.

Two new and closely linked factors have come into play since 1898 and are largely responsible for the present position: the widening of the scope of science in agriculture and the agricultural development of the British Empire and of South America. In the nineteenth century agriculture had been mainly a branch of chemistry; its professors had been chemists, its laboratories chemical. Crookes suggested more chemistry as the way out of what he called the 'colossal dilemma' of world starvation; he proposed the manufacture of more nitrogenous fertilisers from the air—a fantastic idea at the time, yet now our chief source of supply.

The new scientific developments came from the biological side, and the new practical developments from the engineering side. The first great biological triumphs were in plant breeding. There had always been an empirical art of plant breeding and selection which had given to farmers in the nineteenth century the Hallett barleys, Browick, Red Standard and other good wheats, Magnum Bonum potatoes, and sugar-beets of successively higher sugar content; but the results came by accident and not by design. With the discovery of Mendel's laws and the development of the science of plant genetics, the production of new varieties was largely under control; within limits the breeder could work to a specification with considerable hopes of success. The greatest success has been achieved in producing varieties with some special quality such as drought resistance, shortened growing period or stiffer straw; this has proved far more fruitful than the quest for generally improved varieties. For by developing some special quality it has been found possible to cultivate the crop in regions where the older varieties would not grow.

Animal breeding is following the same lines: the empirical work of Robert Bakewell of Dishley, John Ellman of Glynde, the Collins brothers and a host of others, has given us our unrivalled breeds of livestock. Crew and his colleagues at Edinburgh are now introducing the science of genetics into the industry: they have made a promising start: let us hope they will achieve as great results as their colleagues have done with plants.

Canada affords some of the best examples of the plant breeder's success in opening up new regions of the world for settlement. Up to the middle of the nineteenth century the Canadian wheats were suited only to the eastern provinces, Ontario and Quebec; they were uncertain on the prairies. About 1842 David Fife, in Ontario, received for trial from a Glasgow friend several packets of wheat which he sowed. Among the resulting plants was one that differed entirely from the rest, and also escaped damage from rust and frost, two destroyers of wheat in those times. How the seed got there, or whence it came, can never be known. It was a Galician variety. But the accident was a fortunate one for Canada, and did much to build up her wealth. The wheat plant was so good that Fife saved the seed and multiplied it, and in course of time it was widely taken up by farmers under the name of Red Fife. It proved

to be eminently suited to the prairies, and as soon as the railway was completed in 1886 it was taken there by the new settlers and became the basis of their prosperity. So strange an accident could not be expected again, nor did Canada count upon it, yet it happened. The Dominion Experimental Farm was set up in 1886 and its director, William Saunders, began the breeding of new varieties. Many of these, while not sufficiently promising to justify multiplication, were kept alive, and one of them, after ten years of seclusion, was picked out in 1902 by his son Charles, who, regardless of much mild chaffing, applied to all wheats within reach his rapid chewing test for quality. This variety was multiplied, and from 1910 onwards was distributed under the name of Marquis to the prairie provinces and the United States. It ripened earlier than Red Fife and so could be grown further north and west; thus it greatly extended the wheat belt of Canada. But even more good fortune was in store, for its earlier ripening enabled it to escape the worst ravages of stem rust. It has in consequence spread southwards into the United States, and it is now probably more extensively grown than any other variety of wheat in the world.

The Canadian plant breeders continued their search for still earlier maturing varieties; they produced Prelude and Ruby, and now Reward, best of all of them in earliness and in resisting stem rust, requiring only about 100 days from seed-time to harvest, and therefore capable of growing much further north than Marquis. Thus has the plant breeder exploited the first lucky chance that gave the prairies a suitable wheat, and he has produced varieties better and better suited to the northern margin of cultivation, and so has pushed the wheat belt into regions counted as waste in 1900.

Man-power was long the limiting factor in Canadian farming, and this problem of saving labour has been attacked with devastating thoroughness by engineers all the world over. The reaper had come in the 60's, and the binder in the 80's, but the internal combustion engine has made changes vast and dramatic beyond the wildest stretches of the pre-war imagination. The tractor and the new cultivating implements at and before seeding-time, and the combine at harvesting, have revolutionised wheat-growing by dispensing with enormous numbers of men and greatly increasing the area of land needed per man as an economic unit for wheat farming. Not long ago 160 acres was the economic unit for the family farm; now 320 acres is the lowest limit, and 640 acres is nearer the most profitable size. C. W. Peterson in his recent book, 'Wheat,' gives some startling figures. In 1911 sixteen persons were needed on the average to cultivate 1,000 acres of land in the three prairie provinces. By 1926 this number had been reduced to eleven. Further reduction has gone on; during the past two years, he says, mechanisation has displaced over 25,000 men from western farms. Fortunately, there is still land to which they can go, for the new machines and the new varieties have enabled land hitherto unsuitable to be brought into cultivation; between 1911 and 1926 the area under crops had risen in the three prairie provinces from 17.6 millions to 35 million acres. Already Canada has far outstripped the limits set by the experts of thirty years ago, excepting only those of the arch optimist, William Saunders; and no one would now risk his

reputation by predicting the limit to Canada's future accomplishments. The result of the new methods is, according to Mr. Peterson, that wheat can already be produced at 43 cents per bushel, or 14s. per quarter (at 25 bushels per acre), and the cost can be further reduced.

Australia also has developed as the result of the activities of the plant breeder and the engineer; the problem here was the conquest of the drought. Farrer began by producing wheats more resistant to rust and drought than the older sorts, and his pupils, Sutton and others, have continued the work. Agriculturists showed the great value of superphosphates for all crops; they further improved the methods of cultivation, and now, as A. E. V. Richardson has shown, for each inch of rain falling during the season, the farmers of Victoria obtain one bushel of wheat, while forty years ago they obtained only half a bushel; further improvement is possible, for with perfect utilisation of the rain one inch should yield 3.5 bushels of wheat. Every new improvement enables the wheat grower to push the wheat belt a little further into the drier inland region, just as in Canada it enables him to push a little further into the northern regions of shorter summers. Some of the most striking agricultural developments of modern times have been in Western Australia.

South Africa owes much of its advances to two other branches of biological science—veterinary science and parasitology. No part of the white man's habitation seems so suitable for insects, and especially parasites, as South Africa. So long as the white man occupied the country only thinly he could do it without difficulty, but trouble began as soon as he wished to increase his hold on the land and multiply his flocks and herds. The first to attack the problem seriously was Arnold Theiler. It is difficult to overrate the value of the service he has rendered to South Africa as a country, and to farm animals the whole world over. He began at the time of the rinderpest plague of 1895, a virus disease which killed almost the entire cattle population of South Africa; the country was also devastated by horse sickness, blue tongue of sheep, heartwater of cattle, sheep and goats, and other terrible diseases. With almost uncanny precision he diagnosed the causes of these diseases and discovered curative measures; he founded the Veterinary Research Laboratories at Onderstepoort, of which not only South Africa but the whole Empire is proud, and he trained up a body of veterinary research workers and officers who now, under the distinguished leadership of P. J. du Toit, are extending the good work. Dr. du Toit, in his brilliant presidential address to this section last year, set out the history and present position of the achievements in veterinary science. These discoveries have had their counterpart in the veterinary services of India and other countries, and animal diseases are now much more under control than they were. However, the task never ends, for as soon as one disease is controlled another seems to rise into prominence. We are still far from security; in the past twelve years foot and mouth disease has cost the British Government over 5½ million pounds sterling paid to the farmers of Great Britain as compensation for animals compulsorily slaughtered, while the farmers themselves have suffered vastly more. Veterinary research is now developing in this country at Cambridge and elsewhere, and the relationships between nutrition and disease are studied at the Rowett Institute.

The engineer has perhaps been the greatest force in the development of New Zealand agriculture. In 1831, the time of our first meeting, the only export from New Zealand was a little flax (with an occasional preserved human head elaborately tattooed); wool was not exported till 1835, and then only from two farms; there was no organised settlement till 1840, when Wellington was founded, and no real movement till 1843, when numbers of sheep were brought over from Australia and established on the Wairarapa plains near Wellington. Wool rapidly became the chief export, followed for a short time after 1870 by wheat, the result of Vogel's development policy, till the invention of refrigeration paved the way for the great dairy and lamb industries, which are now among the most remarkable and efficient agricultural industries in the world. The invention came from Australia; in 1873 James Harrison had been awarded a gold medal at the Melbourne Exhibition for his method of freezing meat. But the method was not developed till 1879, and then it was not successful. The first satisfactory cargo of frozen mutton and lamb came to London from New Zealand in 1882 in a sailing ship fitted with refrigeration appliances; ten years later steamers were introduced, and continuous improvements have since been made. On the agricultural side also the industry has developed remarkably, and from 1921 onwards it has been the subject of a good deal of legislative control, for the New Zealand farmer has learned to combine freedom of action in producing with united action in grading and marketing, and in consequence he has been able to send over here large and regular supplies of uniform high quality, and so to secure an enviable position in our markets. He does this at a profit in spite of his great distance from our markets, and of having to pay wages much higher per man (though not per job) than are paid here; the exports are rapidly rising. In 1929 that of butter was valued at £13·2 millions, of cheese £7 millions, frozen meat (mutton and lamb) £9·9 millions; in all more than £30 millions by refrigeration transport, as against £15 millions of wool—a truly remarkable progress.

The development of the dairy industry, however, was not simply a matter of transport: it is a triumph for the bacteriologist, who has reduced to an exact science the art of producing clean milk, good butter, and cheese true to type. In this country good work has been done at the Dairy Research Institute at Reading by Stenhouse Williams, Golding and their colleagues.

Australia has recently made great progress with the dairy industry, and is now going into the question of lamb. Canada has a highly developed dairy industry. These new developments require compact units, and therefore intensive farming. The natural herbage, supplemented where necessary by mineral licks, had sufficed so long as wool and low-grade beef alone were produced, but with intensification came the necessity for improving the grazing lands. Treatment with phosphate, which Wrightson, Somerville, Gilchrist and others had shown to do so much for British pastures, proved equally effective in New Zealand, the enclosed paddocks of Australia and parts of South Africa; indeed, few results are more striking than those obtained with phosphate on almost any crop in these countries. These problems are now being studied by Orr and the staff of the Rowett Institute. In the moister areas the striking results obtained

with nitrogenous manures on hay at Rothamsted during the past 80 years have been obtained also on grazing land, and intensive methods such as that proposed by Falke and Warmbold in Germany, and developed by Imperial Chemical Industries, are being tried in this country and in the British Empire. Stapledon has shown the marked differences between different strains of grass. The grass lands of the Empire can be considerably improved, and vast increases are possible in the output of meat and dairy products.

Beef production is in a somewhat different category from mutton or pig meat. In the Norfolk rotation it was linked to intensive farming, but this has long been uneconomic, and it is now moving back to the extensive grassland systems. It does not join up well with the systems of producing dairy produce and mutton practised in New Zealand, Australia and Canada, and it requires different refrigerator arrangements. The future supplies appear at the moment to be less extensive and less extensible than those for other products. There are, however, two great regions of the British Empire where great extension will be possible whenever the need arises: the northern part of Australia, and the grass region of Africa lying between latitudes of 20° South and 15° North—roughly between the Limpopo and the Sahara group of deserts—it includes the Rhodesias, Tanganyika, Kenya, Uganda, Somaliland, the Southern Sudan and the Western Colonies. There are, of course, entomological and veterinary difficulties, for insects are in possession of much of this country; there are also sociological problems, for many of the natives do not wish to sell their cattle, holding them as marks of honour and distinction; there are transport problems and many others; probably none, however, is insuperable.

Another result of improved storage during transport has been a great development of Empire fruit growing. Apples and oranges were formerly obtainable in England only in winter; they are now obtainable in spring and summer, thanks to the marked developments in Tasmania, the Murray region in Australia, and South Africa. Plums, peaches, grapes come in abundance from South Africa, bananas from Jamaica; not only are the total imports of fruit increasing, but the proportion from the Empire increases; it had averaged 24 per cent. for the five years 1925–9, and rose to 33 per cent. in 1930; home growers supplied 26 per cent.; usually their share is nearer 30 per cent. The Empire still, however, supplies less than one orange out of every four that we eat, only 39 per cent. of our bananas, 16 per cent. of our grape fruit, and 10 per cent. of our pine-apples; there are therefore considerable possibilities of further development. Demand is increasing; in 1930 the consumption of fruit per head of population in Great Britain was nearly 83 lbs., as against 70 lbs. in 1924. Other countries are improving their production and transport. In Great Britain, Barker, Wallace, and their colleagues at Long Ashton, and Hatton at East Malling, have greatly strengthened the fruit-growers' position, and for fruit the outlook is, as for other commodities, a power of production growing greater than the power of consumption. Another important factor in the fruit industry has been the development of canning, which affords a satisfactory way of dealing with excess produce.

Engineering science has further intensified agricultural production by

developments in irrigation. This ancient art originated in Mesopotamia and Egypt, and then almost died out. It was then taken up by the Americans and the British, and is now almost an Anglo-American science. The engineer provides the water and the drainage, the agriculturist devises the appropriate system of husbandry, finds the most suitable varieties and the ways of growing them, and shows how to obtain the maximum value for the water used. The soil expert distinguishes those areas that can advantageously be watered from those that should not, and discovers also the effect which the water will subsequently have on the soil, and the interactions likely to occur between the soil and the soluble salts almost invariably present. The plant pathologist deals with the plant diseases that inevitably occur, and the medical authorities must keep a close watch for malaria. It seems a formidable technical staff, but constant watchfulness is imperative; success in the first ten or fifteen years is easily enough attained, but serious troubles sooner or later dog the steps of those who change a natural desert into an artificial garden. *Naturam expellas furca, tamen usque recurret.* You may drive out Nature with a pitchfork, but she always comes back again. The Spirit of the Waste is not too easily conquered.

The greatest triumphs of irrigation in our time have been in India. There British engineers have set up the greatest dams, the greatest canals, the greatest schemes the world can show. The cultivable area of India has been enormously increased, and land provided for millions of peasants who would otherwise have had none. Since the British introduced the great modern schemes famine has been banished from India—not only famine but even the memory of famine and of the self-sacrificing labours of those who finally overcame it. These Indian irrigation schemes are an unmixed blessing; they are largely used for local food production, and they raise the standard of life for the peasants without lowering the standard of life of anyone else by flooding the world market with cheap products. Irrigation schemes worked by white men are so costly that only valuable products can be raised. The Murray River basin in Australia, the largest white man's scheme in the Empire, produces dairy produce, oranges, peaches, raisins and other fruits for the world market, and rice, which largely goes to the East. The main purpose in Western Canada is fruit and dairy produce, in the White River and other settlements in South Africa, oranges. In hotter regions the schemes are worked by natives under British supervision, but usually for costly crops; in the Gezira cotton is the purpose. In all cases irrigation has greatly increased the output from the land and greatly increased the supplies for the world market. If time permitted, it would be possible to go through the whole list of products of the earth and show how modern science has increased output far beyond human needs, with a resulting fall in demand and lowered prices. One could dilate on the achievements of the Dutch in Java in producing their new sugar cane, which quadrupled the output and so lowered the price of sugar that the West Indies are in terrible distress, the sugar-beet industry of this country is threatened, and all Europe would be in trouble but that they artificially keep out the new sugar. Or again, one could speak of the achievements in rubber growing, of the change over from wild rubber to plantation rubber, of the extra-

ordinary improvements in technique, which have in the past thirty years so enormously increased the output that even the most extensive new demands of modern civilisation—rubber tyres, rubber floors—have failed to keep pace with supplies, so that the price, which in 1910 was 12s. 6d. per lb., is now reduced to 3d., and may fall still lower, causing great distress to the rubber growers.

Modern science, in short, has been so successful in increasing man's power over Nature that it has brought us harvests far more bountiful than we know what to do with. Science is still advancing, and no one can tell what it will achieve next.

In these circumstances, with this plethora of the products of the soil, with these gifts of Nature poured upon us not merely bountifully but torrentially, so that many of our farmers are likely to be submerged in the process, one might well be tempted to ask should not the scientific workers halt for a time? It sounds a reasonable question and it is easily answered: they cannot do so even if they wished. Their purpose is to gain knowledge of Nature, especially of soils, crops, animals and their relations to one another, and in this quest there can be no halting. Three reasons will suffice. The march of civilisation is inextricably bound up with the search after knowledge, and all history shows that, when intellectual advancement ceases, civilisation rapidly comes to a standstill. The pursuit of knowledge is a human necessity; it is part of our make-up, and we owe to it much of what dignity we possess. We could no more suppress it than we could suppress human emotions or physical needs. Secondly, the knowledge so gained furnishes the only possible material for agricultural education. Empiricism alone is never a sound basis; it may arouse, but it never satisfies, intellectual curiosity, and it does not open up those vistas of promising investigation which a well-designed experiment so often reveals, the exploration of which calls forth and develops some of the finest intellectual qualities in mankind. The necessity for agricultural education is now universally admitted; only the intelligent, mentally alert, well-trained farmer has much chance of success; and one cannot have agricultural education without constant research to test and expand the body of knowledge which the teacher imparts, ruthlessly cutting out anything false or unfounded.

And lastly, although we may think in our pride that we have achieved a wonderful control over Nature, yet our control is really very limited, our tenure uncertain, and our margin of safety very exiguous. Crookes' disquieting forecast of 1898 failed to eventuate not because it was false, but simply because new powers were won by mankind in the form of plant genetics and the internal combustion engine. How long mankind will have the wit to go on developing more powers we do not know; human activities hitherto have gone in cycles, and it may be that the period of scientific activity is nearly ended. It is quite certain that any slackening of control or failure to utilise scientific discovery by any one group of cultivators would speedily eliminate them through pressure of more enlightened and therefore more successful competitors. It is, however, not so much human competition as the opposing natural agencies that must continuously be watched. The weather can still defeat our best laid farming plans. Irrigation schemes, however impressively they seem



to conquer the waste, are always liable to fail through soil troubles, plant diseases or insect attacks. Over large parts of our Empire there is a continuous struggle for possession between insects and men, and the margin of victory, even when we get it, is never very great. And there are new troubles as yet only dimly seen that may easily cause great difficulty in future. The remarkable development of rapid transport has carried all over the world not only the blessings but also the evils of this earth. Pests and diseases of animals, and particularly of plants, have only to appear in one corner of the globe to spread elsewhere with great rapidity despite all regulations to the contrary, often causing enormous losses. Among the most serious troubles of modern times are the virus diseases of plants. These diseases are apparently not caused by any recognisable living organism, nor are they simple physiological disturbances; they cannot yet be attributed to any definite causal agent. They spread rapidly, being frequently carried by small insects, sometimes by mere contact, and they cannot be cured, one can only stand by and see the plants perish. All kinds of crops are affected: sugar-cane, tobacco, cotton, sugar-beet, groundnuts, bananas, potatoes, maize, timber trees (*e.g.* Sandal), large and small fruits (*e.g.* peach and raspberry) and most greenhouse and horticultural plants. And it is not so much sickly plants as healthy ones that suffer; the disease may come suddenly and with great virulence into a healthy prosperous region and devastate the most important crop. In Gambia the Rosetta disease cut down the crop of groundnuts to about one-third of the normal yield.<sup>7</sup> In the United States in 1926 two virus diseases reduced the crop of potatoes by no less than 16 million bushels. In this country the total loss cannot be estimated, but the figures recorded for various attacks vary from 35 to 75 per cent. loss of crop. Worse still is the deterioration of stocks: stocks apparently healthy and vigorous may become worthless in two to four years. Cotton growers are becoming seriously perturbed. In the Gezira last year the losses were considerable, although until recently the leaf-curl disease was unknown there. Sugar-beet in the south-western region of the United States is so seriously imperilled by the curly-top disease that the Government has set aside \$300,000 for its investigation. In this country special grants are made to Rothamsted, Cheshunt, Bangor, and other institutions to study these diseases. Tobacco is now being badly attacked, also tomatoes and potatoes; the latest sufferers are the narcissi and daffodils in our own gardens; these cease to flower and shortly perish. Virus diseases are quite recent as serious plagues; if they are old they have hitherto been unimportant or unnoticed. Clearly Pandora's box is not yet empty.

Now a cynic might say that it is no bad thing thus to discover a way of making one blade of grass grow where two grew before. If these troubles affected only certain areas or groups of growers the more fortunate producers might regard them with sympathetic equanimity. Unfortunately, however, they may befall any farmer, good or bad, and the better the farmer the greater the loss may be. Plant pathology has not yet had its Arnold Theiler to show the way of insuring health to farm crops,

<sup>7</sup> Gambia Report, 1925.

nor has it had the success won by the fruit investigators in dealing with their pests.

It is indeed not less knowledge but more knowledge that we want. Every country now recognises this. The United States stands easily first in elaboration of agricultural research, organised not only by the Government but by private endowment. Both in England and in the United States men who have made fortunes in the city have spent their money in developing agriculture or agricultural science—following the advice given by one of Plato's people—having acquired wealth, begin to practise virtue. But there has been this interesting difference. The American patron has spent his money on a college or research station, setting up a laboratory or some other new building, or endowing fellowships, so that a succession of vigorous young people could develop the subject, adding also greatly to their own value as workers for agricultural progress. So the gift has fructified and enriched the community in ever-widening circles. The British patron, on the other hand, has usually spent his money on his own estate, making his own experiments in farming. Some have rendered service by carrying pedigree livestock over periods of depression when the commercial farmer might perforce have had to let them go. But many have simply experimented on no very definite basis and with none of the continuity essential to the success of agricultural investigation. While no doubt getting much amusement out of it themselves, they have not achieved results commensurate with the time and money expended, and in any case their successors promptly stop the whole enterprise, whether good or bad, so that the work soon passes out of memory. Without disputing the inalienable right of the Englishman to spend his money in any way he may think fit, and remembering, too, that the pursuit of agriculture is one of the most honourable ways in which a man can lose money, we can still commend to the English patron the wonderful possibilities of the endowment of agricultural research. To say nothing of Lawes and Rothamsted, think what the world has gained through John Quiller Rowett's gift in 1920 of land near Aberdeen, and of £10,000 to erect buildings, thus founding the Rowett Institute, and how much poorer the world would have been had he simply, like many another man of wealth, spent that money in so-called farming experiments. We in England are proud to think that he was an Englishman. Scotland has recently had a further benefaction in the Macaulay Soil Institute set up to study the peat soils of Scotland and to help the farmers there so long as any men farm in Scotland. We remember with gratitude, and we know that our children will do so, the names of Molteno, William Dunn, Thomas Harper Adams,<sup>8</sup> Charles Seale-Hayne,<sup>9</sup> John Innes for their foundations in this country; Peter Waite and John Melrose for the Waite Institute in Australia, William Macdonald for the Macdonald College in Canada,<sup>10</sup>

<sup>8</sup> Left £26,640 in 1892, but this was allowed to accumulate till 1900, when it was worth about £40,000; the college was then built.

<sup>9</sup> This gift of £141,443 was left in 1903, the college was built in 1914, and formally opened in 1919.

<sup>10</sup> This gift of 8 million dollars in 1904 was only part of Sir William's benefaction for Canada.

Thomas Cawthron<sup>11</sup> for the Cawthron Institute in New Zealand. To-day the need is not so much for new Institutions as for the strengthening of some of those already in existence.

Agricultural science has now widened so much that it is co-extensive with the whole range of science, and this has necessitated considerable expansion of staffs and full interchange of ideas and knowledge between the workers. This has proceeded in two different directions.

Within the Empire all agricultural experts are now in touch with the central clearing houses in Great Britain, the Imperial Agricultural Bureaux, whose function it is to search the world for information likely to be useful and then pass it on to the persons likely to want it. These bureaux were set up at the request of a conference called as the result of the address delivered from this chair in 1924. The system is working well.

World organisation of scientific investigation is proceeding rapidly. It is done on the basis of subjects; its method is the holding of international conferences of the technical and scientific experts who to-day control the machine that works the material part of our civilisation.

Of the three factors involved in the agricultural situation—production, marketing and the scientific advisory and technical system—the last is by far the best organised.

Much has recently been done, however, in developing better and more efficient marketing by the Empire Marketing Board and the Ministry of Agriculture. Happily there is a good demand for high quality produce, for small young animals not too fat; as our civilisation advances the expectancy of human life increases, but that of the farm animals decreases. One difficulty is the elusive British housewife, for whom all this elaboration of effort is made. In the main she knows little about the food she buys, and, having glanced at the bewildering display, she usually chooses whatever is cheapest or gives least trouble—not because she is idle, but because in these days it is impossible for her to get domestic help. So there is a great increase in consumption of tinned and preserved foods, of margarine and of imported chilled or frozen meat; the consumption of fresh food shows no increase per head of population, while that of preserved food does. One of the needs of the day is a definite experimental inquiry to find out whether the freshness of food, of which the British farmer has almost a monopoly, is or is not an advantage to the consumer. So far we have only the Scottish experiments which showed the superiority of fresh over pasteurised milk.

Our greatest need, however, is a better organisation of agricultural production. A beginning has been made by the overseas farmers; the necessity for sending all produce through one or two ports has compelled them to work through large organisations for grading, transporting and selling the produce, with skilled representatives in this country. Dealing in hundreds or thousands of tons they reduce all costs and all wastage to a minimum. Gradually the British farmer is organising; the difficulty is to do this without destroying his sturdy individuality, one of his greatest assets, the loss of which would irretrievably damage our country life.

But greater organisation is possible and is highly desirable.

<sup>11</sup> Born in Camberwell 1833; died at Nelson, N.Z., 1915.

At present British farmers, Empire farmers, and farmers from all over the world indulge in deadly competition in the British market. In the end they obtain wholly inadequate prices. But the community as a whole does not gain because they lose. The final cost of food to the consumer is profoundly affected by costs of handling, transport, preparation and distribution, all expensive services. Better organisation of production, while benefiting the countryman, would not injure the rest of the community.

Thanks to the inquiries made by the Ministry of Agriculture and the Empire Marketing Board, the food requirements of this country are pretty well known. Our next great step forward will be to organise production on a contract basis so as to satisfy these requirements with a reasonable margin of safety, but without the terrible waste involved in those large excesses which injure the grower without benefiting the consumer.

Something of the sort is essential if farming is to survive as an occupation for the best of our people, offering a reasonable standard of living to farmer and worker. The advantages would be incalculable. Organised production and the development of the contract system which has done so much for the milk producers, would permit of a renewal and development of country life to the fullest extent now made possible by scientific and technical advances. By common consent many of the ills of to-day arise from the fact that for nearly a century the industrial side of our national life has been fostered at the expense of the rural side, producing an over-industrialised town population peculiarly susceptible to world economic disturbances, and now largely without employment or prospect of employment. The rural population, on the other hand, is far less sensitive to economic disturbances; the low rate of unemployment in the countryside shows the greater independence and resilience of the conditions of country life, and points clearly to the fact that improvements in our rural life would benefit not only the countryman but the whole community.



# The Effect of Climatic Conditions on the Growth of Barley.

BY

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With six Figures in the Text.

THE relation of climate to the growth of the plant has important bearings on many problems of scientific as well as economic interest. It is, for instance, impossible to interpret the effect of manures on crop yield by an experimental method consisting of repetitions year by year of field trials, unless variations in climate are taken into account in assessing the results of such experiments.

The production also of new varieties suitable for local climatic conditions must always remain a matter of pure empiricism, until the interaction of the climatic complex with the physiological processes of distinct races is understood. Furthermore, the application of physiology to agricultural problems seems to the author to find its most fertile field in disentangling the effect of external factors from the general interplay of processes determining the yield of the plant. It may confidently be expected that in this way the necessary experimental evidence required to check knowledge acquired by laboratory methods, where external factors are as far as possible varied one at a time, will be obtained.

Investigations of this kind are singularly lacking, in spite of the fact that this aspect of physiological problems led to a considerable deal of work in Germany, mainly between 1877 and 1880, under the inspiration of Kreuzler. A great mass of data was at this time accumulated, but methods of dealing with the experimental results had not then been developed, and until recent years the information thus collected was valueless to science.

The subject has been reopened recently, and a paper dealing with the problem on these lines was published in 1920, embodying the analysis by R. A. Fisher, in collaboration with W. E. Brenchley, of data on the growth of *Pisum sativum*.

The author has been interested since 1917 in problems of this kind, and in that year published in the Report of the Cheshunt Exp. Station an outline of a method of analysis of plant growth, together with figures of net assimilation rates calculated from dry weight and leaf-area data. The method there developed has since been employed and elaborated by Kidd, West, and Briggs.

#### EXPERIMENTAL METHOD.

A pure line Goldthorpe barley has been grown in pot culture at Rothamsted each year since 1921. The soil used was obtained from one of the experimental fields (Little Hoos) and before use was sieved and mixed with 10 per cent. of sand. The pots were of glazed earthenware, measuring 10 in. by 10 in., holding approximately 30 lb. of soil. They were provided below with a tubulure which was closed with a cork carrying a glass tube with a right-angled bend leading into a glass vessel to catch drainage water. At the time of filling the pots, the water content of the soil was determined and exactly 30 lb. of soil were weighed into each pot. Sufficient water was then added to bring the water content up to 15 per cent., which was known to be optimum for this particular soil. Throughout the experiments the aim was to maintain the water content at this figure, and although higher water contents could not be prevented, since the pots were exposed on the roof of the laboratory, 15 per cent. represented the lower limit. To ensure this end, each pot was weighed twice a week during dry weather and the water lost by evaporation was replaced, either from a tank of tap-water kept at air temperature or from the bottles of 'percolated' water.

The seeds before planting were first selected by eye for uniformity and then graded by weight between the limits 50–60 mg. Six seeds were sown in each pot, of which three were subsequently removed, leaving the three most uniform seedlings. For reasons to be explained subsequently, two types of manurial treatment were used, *A* consisting of 0.5 grm.  $\text{NaNO}_3$ , 1 grm. superphosphate, 0.25 grm.  $\text{K}_2\text{SO}_4$  per plant, and the other, *B*, differing in having 1 grm.  $\text{NaNO}_3$  and 1 grm.  $\text{KNO}_3$  per plant. In spite of the manurial differences all the series of experiments have been utilized for the purpose of this paper. This was possible since, as will be seen later, the assimilation rates in the two series were identical, and correction was made for the final differences in dry weight and leaf-area.

The manurial treatments and phenological data are given in the following table:

TABLE I.

Year.	Manurial Treatment.	Sowing Date.	Germination Date.	Harvest.
1921	A.	May 3	May 10	Aug. 9
1922	A.	May 8	May 17	Oct. 2
"	B.	May 16	May 22	Oct. 2
1923	A.	May 1	May 7	Sept. 12
1924	B.	April 16	April 23	Aug. 25

Six pots were removed each week, the soil washed out from the roots by a stream of water, the leaf-area measured with a planimeter, and the plants subsequently dried in an electric oven at 102° C. Before drying the plants were separated into roots, stems (including leaf sheaths), dead leaves, green leaves, and ears, each of which were weighed separately. The dry material thus obtained was afterwards ground and used for determination of total nitrogen, ash, and calorific value. These data will be considered in a subsequent paper. The considerations here put forward will deal only with leaf area and data of total dry weight.

The primary plant data thus collected provided information on the dry weight and leaf-area of the average of eighteen plants, week by week throughout the growing season. It is not necessary here to discuss fully the accuracy of these data, but it may be said that the probable error of the average dry weight rarely exceeded  $\pm 2$  per cent., and for many samples was below  $\pm 1$  per cent. The data for leaf-area were somewhat less reliable, as in later samples it was found impossible to measure more than three individual plants, and the mean leaf-area had to be calculated from the relation established between leaf-area and leaf weight. From these primary data the net assimilation rate was calculated in a manner to be explained later.

#### ANALYSIS OF THE DATA.

For purposes of analysis three measures of growth have been used :

- (1) Net assimilation rate.
- (2) Relative growth rate of the leaf surface.
- (3) Relative rate of increase in dry weight. (Efficiency index.)

The meteorological data with which these have been correlated were collected *in situ*, and consisted of average day temperature, average night temperature, total solar radiation, hours of bright sunshine, and the evaporating power of the air. The temperatures were derived from continuous thermograph records by averaging two-hourly readings on the charts for day and night throughout the season. Radiation was measured with a Callendar self-recording radiometer, the record for each day being



integrated with a planimeter. The evaporation data were obtained from a porcelain atmometer belonging to the Rothamsted Experimental Station, fully exposed to rain on the laboratory roof; hence these last data can scarcely be considered satisfactory.

#### CLIMATIC CONDITIONS.

The variation of climate encountered during the course of the experiment is considerable. 1921 will long be remembered as a year of continuous sunshine and lack of rain. 1922 was characterized by a spell of fine weather immediately after germination, giving way later to almost continuous dull and rainy weather. In 1923 and 1924 conditions were almost the antithesis of these, the weather at first being dull and cold and improving later. Full details of the climatic conditions are recorded in the following table :

TABLE II.

Month.	Max. Temperature.				Min. Temperature.				Hrs. Bright Sunshine.				Rainfall (inches).			
	1921	1922	1923	1924	1921	1922	1923	1924	1921	1922	1923	1924	1921	1922	1923	1924
May	62.1	65.4	56.7	61.1	43.3	44.7	42.0	45.4	209	280	166	191	1.445	1.579	1.681	4.628
June	67.5	65.9	60.7	65.2	47.6	48.1	46.8	50.2	216	229	116	200	0.194	1.038	0.617	1.974
July	77.0	63.7	72.5	68.3	53.4	49.7	55.1	51.0	240	150	224	236	0.179	4.605	3.871	4.533
August	69.2	63.2	68.5	64.8	52.6	49.2	51.0	50.5	145	127	257	169	1.113	2.930	2.329	2.351
September	—	60.5	62.9	—	—	46.3	46.2	—	—	103	189	—	—	2.882	2.541	—

In dealing with the complex relations between the measures of growth and the changing environmental conditions, the ordinary method of drawing graphs indicating the relation between the variables fails, since it is obvious that over periods as long as a week all the factors have been conditioning the rates of the processes, and the effects of the individual factors obscure each other. The relations sought for can, however, be determined by the method of correlation. The assumption is made that, had the magnitude of all environmental factors remained at the mean value, the rate of the process studied (net assimilation rate, relative leaf growth rate, &c.) would have remained at its mean value for the whole period. Any change in the rate of the process is assumed to be due to the change from their mean values of one or more of the external factors. The method deals with these departures from the mean values, and investigates the extent to which they are associated.

Difficulties immediately arise :

1. The external factors themselves do not vary independently, as, for example, solar radiation and temperature-level tend to vary together. By taking 'partial correlations' this difficulty is overcome, and allowance is made for the relations inherent among the environmental conditions, in assessing the effect of each, singly, on the rate of the process studied.

2. The assumption is made that, in so far as variations in the level of the external factor affect the rate of the process at all, small or large variations will have proportionate effects. This is by no means true, as an optimum value for external factors acting in combination undoubtedly exists. In spite of this, however, the trend of the interrelations within the range of conditions explored will appear from the analysis, unless any of the factors have an optimum at the mean value, in which case the partial correlation coefficient may be zero and the rise and fall in the rate of the process will be obscured. Also, clearly, it will be inadmissible to extrapolate outside the range of values actually explored.

3. Even were the external factors to remain constant at their mean values, the rate of such a process as relative dry weight increase would not remain constant at its mean value. In fact, the rate of this process is not solely conditioned by external factors, but depends also on the action of varying internal factors which independently determine the rates at successive moments. The method devised to overcome this difficulty will be described later. The net assimilation rate does, however, appear to fulfil the stipulated condition.

It is necessary to stress first of all the importance of the numerical measures selected for evaluating the external factors. It is desirable to use quantities which are easily measured, and for this reason maximum and minimum temperatures and hours of bright sunshine are ideal measures of climate, and indeed are the characteristics generally selected for meteorological records. For physiological purposes they are singularly unsatisfactory. An indispensable characteristic of climatological indices is that the distribution in time of the intensity of the factor measured shall be included in the standard. Integrated thermograph records, or, what is almost equivalent, averages of two-hourly readings of the chart, fulfil this requirement, as do also integrated records of the Callendar radiometer, and the values for radiation derived from the latter share with the former the advantage that all intensities are recorded, and not maximal intensities only, as in records of bright sunshine.

It is not the author's intention to discuss the whole question of climatological indices in this connexion, but it may be pointed out that Livingstone's<sup>1</sup> suggestion of using averages of temperature, weighted according to growth rates of specified plants observed under laboratory conditions, cannot simplify the problem of determining the relation of plant growth to the climatic complex and the part played therein by the temperature factor. It is precisely the change in the relation of growth to the single factor, brought about by the interaction of other factors, that needs to be known, and

<sup>1</sup> B. E. Livingstone : *Temperature Coefficients in Plant Geography and Climatology*. Bot. Gaz., lvi, pp. 349-75, 1913.

can only be derived from the primary data. It must be realized that it is not necessary in the calculation of correlations to restrict oneself to the first power of the variables, such as temperature, and that by utilizing higher powers, such as  $t^2$  or  $t^3$ , &c., and treating these as independent variables for calculating partial correlations, the relation between the rate of the process studied and external factors may be expressed with any degree of nicety, and the regression lines may be curves of any form. In this way optima may be located with a degree of precision depending only on the patience of the computer and the quantity of data available. A paucity of data alone made such treatment in this case unprofitable.

### *The Interrelation of Climatic Factors.*

The mean values for the climatic conditions during the periods of the experiments are summarized below :

	<i>Mean.</i>	<i>Standard Deviation.</i>
A. Mean max. temp.	64.94° F.	4.567° F.
B. " min. "	47.94° F.	3.259° F.
C. Mean hours sunshine per week	44.76 hours	18.026 hours
1. Average day temp.	57.08° F.	3.858° F.
2. " night "	52.07° F.	3.033° F.
3. Total radiation in cal. per sq. cm. per week	2513 cal.	507.4 cal.
6. Evaporating power of the air (grm. evapo- rated per hour)	0.4321 grm.	0.2915 grm.

The extent to which these factors are interrelated is shown by the following correlation coefficients, calculated from thirty-one pairs of values :

$$\begin{array}{l|l}
 {}^1r_{AB} = +0.590 \pm 0.119 & r_{12} = +0.916 \pm 0.029 \\
 r_{AC} = +0.676 \pm 0.099 & r_{13} = +0.640 \pm 0.108 \\
 r_{BC} = +0.057 \pm 0.182 & r_{23} = +0.425 \pm 0.150
 \end{array}$$

Maximum day temperature and minimum night temperature are much less closely correlated than are average day and average night temperature, though in both cases the factors vary together. The reason for the large difference in correlations between radiation and minimum temperature becomes clear from the partial correlation as shown below.

$$\begin{array}{l|l}
 r_{ABC} = +0.750 & r_{12.3} = +0.926 \\
 r_{AC.B} = +0.797 & r_{13.2} = +0.692 \\
 r_{BC.A} = -0.575 & r_{23.1} = -0.525
 \end{array}$$

${}^1r_{AB}$  signifies the correlation between the two variables *A* and *B*. When partial correlations are used the first two symbols preceding the point represent the two variables under consideration, while the symbols following the point indicate the factors whose effect has been eliminated. Thus  $r_{12.3}$  indicates that four factors have been studied, and represents the correlation between the two factors *A* and *B*, when the effects of *C* and *D* have been eliminated.

When the effect of the third factor has been allowed for, the correlation between day temperature and night temperature rises, while the true relation between night temperature and radiation is found to be inverse; in other words, after allowance has been made for the tendency of warm nights to follow warm days, and sunny days to be associated with high temperatures, it is seen that sunny days tend to precede cold nights. This is undoubtedly due to the absence of cloudiness leading to rapid heat loss at night by radiation. Correlations similar in sign were found to hold in the greenhouse by Dr. W. E. Brenchley.<sup>1</sup> The closer association of maximum temperature with hours of bright sunshine than average temperature with total radiation is to be expected, since a high maximum temperature may be reached even during a short period of bright sunshine, which will not greatly affect the average temperature over the day.

The partial correlations with evaporating power of the air are given below :

$r_{16\cdot23} = +0\cdot429$	1. Average day temperature.
$r_{28\cdot13} = -0\cdot373$	2. Average night temperature.
$r_{36\cdot12} = +0\cdot212$	3. Total radiation.
	6. Evaporating power of the air.

As would be expected, high temperature is associated with high evaporation, but the relatively large effect of high radiation, even after correction for associated temperature, is less easy to interpret, as is also the inverse relation with night temperature. Undoubtedly both exert their action through associated rainfall. The evaporimeter used was not screened from the weather, and hence rain reduced the amount of evaporation registered, apart from the higher humidity prevailing. The positive partial correlation with solar radiation thus indicates the absence of rain at such times, and the negative partial correlation with night temperature signifies only that cloudless nights tend to be colder and are free from rainfall.

### *The Effect of Climatic Conditions on Net Assimilation Rate.*

'Net assimilation rate' may be defined as the increase in weight of the dry material of a plant per unit leaf-area per unit time (unit leaf rate, Briggs, Kidd, and West<sup>2</sup>), and is calculated from the difference in dry weight of average plants at the beginning and end of a given period of time, divided by the average area of the leaf surface during the period. Where observations of leaf-area are made at frequent intervals between the times of sampling, the mean area can be ascertained readily by integrating the curve

<sup>1</sup> W. E. Brenchley: On the Relations between Growth and the Environmental Conditions of Temperature and Bright Sunshine. *Ann. App. Biology*, vol. vi, pp. 211-44, 1920.

<sup>2</sup> C. West, C. E. Brigg, and F. Kidd: Methods and Significant Relations in the Quantitative Analysis of Plant Growth. *New Phytologist*, xix, pp. 200 et seq., 1920.

of leaf area drawn through the experimental points. The net assimilation rates obtained in this way for cucumbers were published by the author in 1917.<sup>1</sup> When information is confined to areas obtained from samples at the beginning and end of a given period of time, the problem of ascertaining the mean area during the period is more difficult. The mean area will clearly be the increment in area divided by the mean relative leaf growth rate.

The problem of determining mean relative growth rates in such cases has been fully dealt with by R. A. Fisher,<sup>2</sup> who has indicated the general method of calculation. The mean areas were obtained by dividing the difference in area at two successive times of sampling by the difference of their napierian logarithms. From each successive pair of samples the net assimilation rates were calculated by dividing the increment in dry weight by the average leaf-area obtained by the above method.

The first part of the whole growth period alone was used for calculating assimilation rates, namely, until the maximum leaf-area had been attained. The reasons for confining attention to this period only are that, firstly, after this time the leaves begin to die off rapidly, and to discriminate between functionally green leaves and dying leaves becomes progressively more difficult; secondly, the stems after this point begin rapidly to elongate and an increasingly large part of total assimilation may be due to their activity.

The factors involved in the analysis are:

1. Average day temperature.
2. Average night temperature.
3. Total radiation.
4. Net assimilation rate in grm. per sq. dm. per week.

$$r_{14 \cdot 23} = +0.394, \text{ significance } 37:1.$$

$$r_{24 \cdot 13} = -0.434, \quad \text{,,} \quad 50:1.$$

$$r_{34 \cdot 12} = +0.429, \quad \text{,,} \quad 50:1.$$

The partial correlations of average day temperature with assimilation rate and total radiation with assimilation, after correcting for the effects of the other two factors, are positive, but the effect of radiation predominates over that of temperature. The negative sign and the magnitude of the correlation with average night temperature are striking. From the fact that net assimilation rate takes no account of respiration, and that the latter increases with average night temperature, while the former is in abeyance, an inverse relation with average night temperature would be expected, but the magnitude of the effect is greater than would be inferred from this, since the length of the night period during the summer is so much shorter than the day.

<sup>1</sup> F. G. Gregory: Third Annual Report, Experimental and Research Station, Chesbunt, 1917.

<sup>2</sup> R. A. Fisher: Some Remarks on the Methods formulated in a Recent Article on 'The Quantitative Analysis of Plant Growth'. *Ann. App. Biol.*, vii, pp. 367-72, 1921.

Partial correlations have also been calculated with maximum day temperature (*A*), minimum night temperature (*B*), and hours of bright sunshine (*C*), and are as follows :

$$r_{A,BC} = +0.544, \text{ significance } > 100:1.$$

$$r_{B,AC} = -0.500, \text{ significance } > 100:1.$$

$$r_{C,AB} = +0.182, \text{ not significant.}$$

The signs of the correlations are similar to those already given, but a striking change in value of the partial correlation with radiation appears. Radiation as measured in terms of hours of bright sunshine is much less closely associated with assimilation than when all intensities are taken into account. This may indicate one of two possibilities: either (1) assimilation rate has an optimum point below the highest intensities of radiation, measured as bright sunshine, or (2) the assimilation at low intensities of light and in diffuse light appears in these correlations as due to temperature effects with which they are correlated, and which have not been allowed for since such light intensities are not included in the measurements in the absence of bright sunshine. Assimilation will certainly proceed in the absence of bright sunshine, and all this will be credited to the concomitant temperature. The important bearing of the choice of standards of measurement of climatic factors appears very clearly in this connexion. The strong adverse effect of high night temperatures again appears in this set of correlations. The regression equation for the first set of partial correlations is stated below :

$$R_n = 0.0499 T - 0.0598 t + 0.1823 L + 0.3551,$$

where *T* is the average day temperature in degrees F., *t* the average night temperature, and *L* is total radiation in 1,000 calories per week. By substituting values of the three variables the net assimilation rate (*R<sub>n</sub>*) may be calculated for all combinations.

Fig. 1 represents the experimental and calculated values of assimilation rate for all the experiments. The agreement all over the range of conditions encountered is very striking. The horizontal line represents the mean assimilation rate of 0.546 grm. per sq. dm. per week, and it will be seen that the experimental and calculated values oscillate together about the mean value. The only large discrepancy is the final value for 1924, and corresponds with a sudden change in climatic conditions. After a period of dull weather with much rain, the weather suddenly became bright and hot, as is indicated in the sudden rise in the calculated value for assimilation. The plants had developed very large leaf surfaces, and the increased evaporation under more favourable conditions may have led to a condition of considerable water-strain. In addition to this, the soil had been saturated with

water for a considerable time, and the lack of aeration may have played a part. In the subsequent week the assimilation rate rose to its calculated value, and it is interesting to note that the dry weight per unit area of leaf rose from 0.338 to 0.352, indicating a rapid thickening of cell-wall. Over 80 per cent. of the variation in assimilation rate is accounted for by change in climatic conditions. Since the effects of other factors have been neglected, and also the effects of sampling error, the agreement with expectation is excellent. As leaf-area and dry weight are highly correlated in individual plants, the sampling errors introduced will tend to be small. It is remarkable that the effect of the omission of two important factors, namely, time and the quantity of nitrogen added as manure, in the analysis should be so small. As regards the first, there is no evidence that, up to the point in the life-cycle at which maximum leaf-area occurs, the net assimilation rate falls off with time, and, should there be such an age effect, it must be very small compared with the changes in assimilation with external conditions. Internal factors apparently play a negligible part in the regulation of the net assimilation rate.

With regard to the effect of nitrogenous manuring, the result is of great interest. Comparing the curves A and B for 1922 (Fig. 1), it is seen that the actual rates found experimentally agree equally well in the two cases with the calculated values, made on the assumption that nitrogen manuring has no effect on assimilation rate, although the quantities of nitrogen added differed by nearly 400 per cent. The maximum leaf-areas obtained, the final dry weights, and tiller numbers in the two cases, on the other hand, differed widely. From the first also a constant percentage difference of nitrogen was observed in the tissues of the plants in the two sets. Hitherto the mechanism by which nitrogen brings about an increase of final yield has not been clear. The larger leaf surface and deeper green colour with increasing nitrogen had been observed, but whether a change in assimilation rate takes place, or whether the larger yields were only due to a larger assimilating surface, was unknown. These results indicate strongly that, over the range of nitrogen manuring here employed, the whole effect on yield is due to the stimulating action of nitrogen on leaf growth, enabling greater total assimilation to take place without change in the net assimilation rate. If real assimilation is increased, then respiration must be correspondingly increased, or the net assimilation would not remain unaffected. The possibility of a very low level of nitrogen manuring depressing assimilation rate has been borne in mind, and experiments to test this point are in progress. In concluding this part of the subject, it may be said that net assimilation rate is almost completely controlled by the factors of temperature and radiation, and internal factors play a minor part. It must, however, be remembered that in these experiments the water relations have been more or less controlled, and in nature their part may sometimes be very significant. Control of

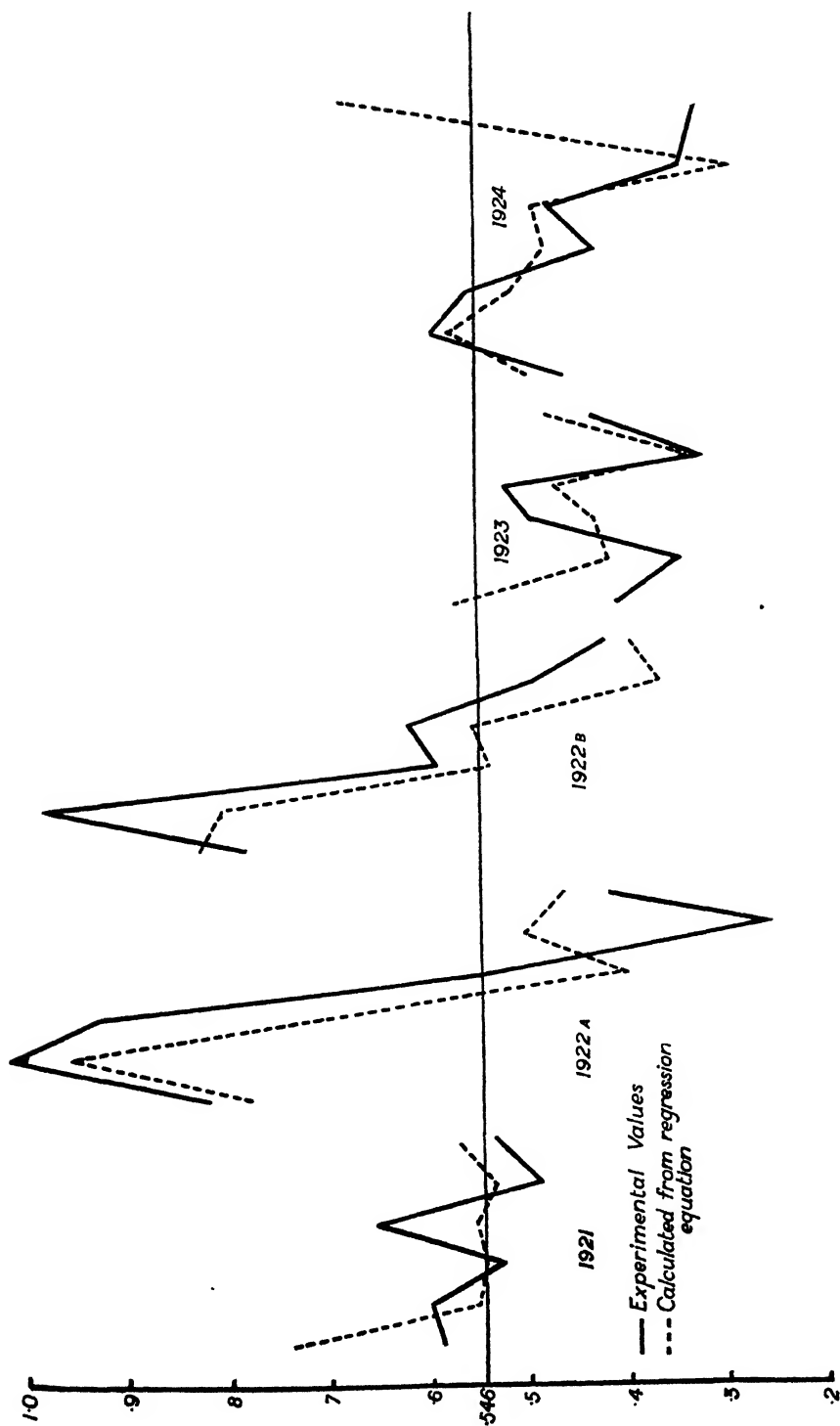


Fig 1. Comparison of experimental values of net assimilation rate with those calculated from regression equation.



net assimilation by temperature and light is almost equally close, and in general neither one nor the other can alone be limiting the process over any considerable period of time.

*The Effect of Climatic Conditions on Relative Leaf Growth Rate.*

The definition of relative leaf growth rate has already been given, and the method of calculation has been indicated. The shape of the curve of

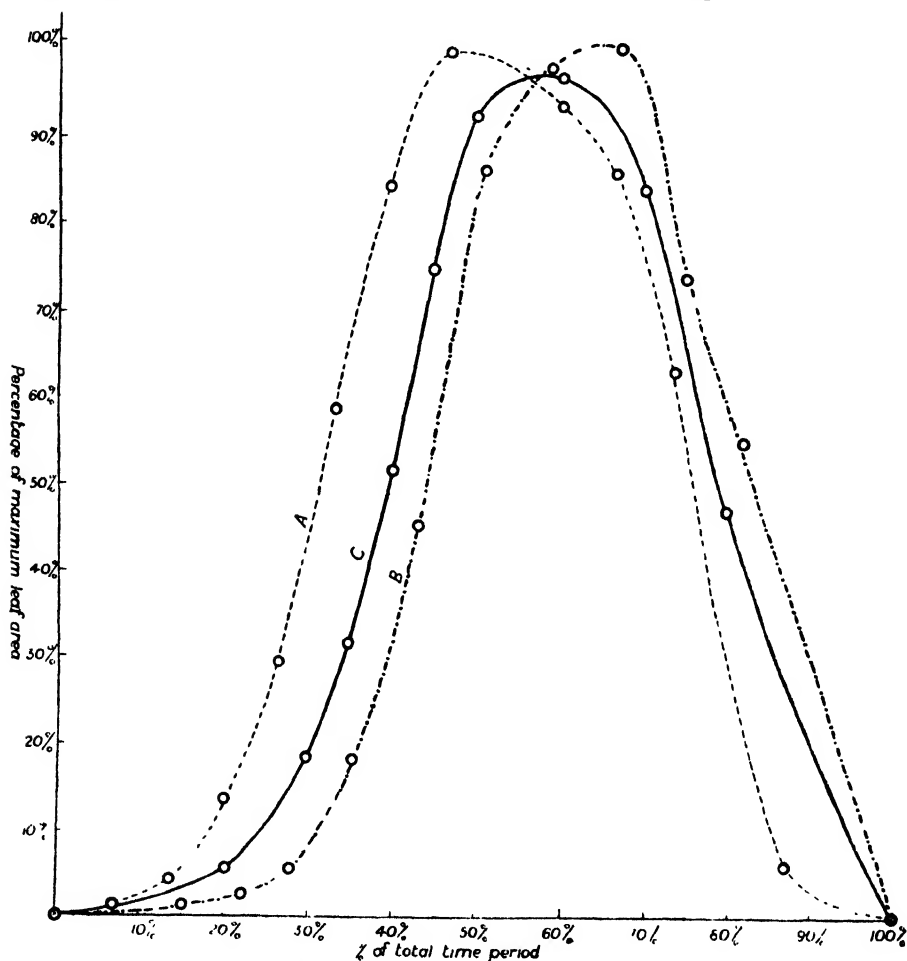


FIG. 2. A. Curve of total leaf-area for Experiment B of 1922. B. Curve of total leaf-area for Experiment of 1924. C. Mean curve (normal curve).

total leaf-area from the beginning up to the death of the leaves is shown in curves A and B, Fig. 2. The curve is of a complex shape, the elucidation of which is reserved for treatment in a subsequent paper. It is, however, obvious that the leaf-area is not a simple function of time, and the relative leaf growth rate varies in time in a complex manner. This precludes the

possibility of calculating straightforward correlations with time as one of the variables, since this would assume a linear time relation. By taking  $t^2$  and  $t^3$  as independent variables, this difficulty could be surmounted, although with great increase in labour. Other difficulties to be mentioned below would, however, still defeat the end.

As the same difficulty is met in dealing with relative dry weight

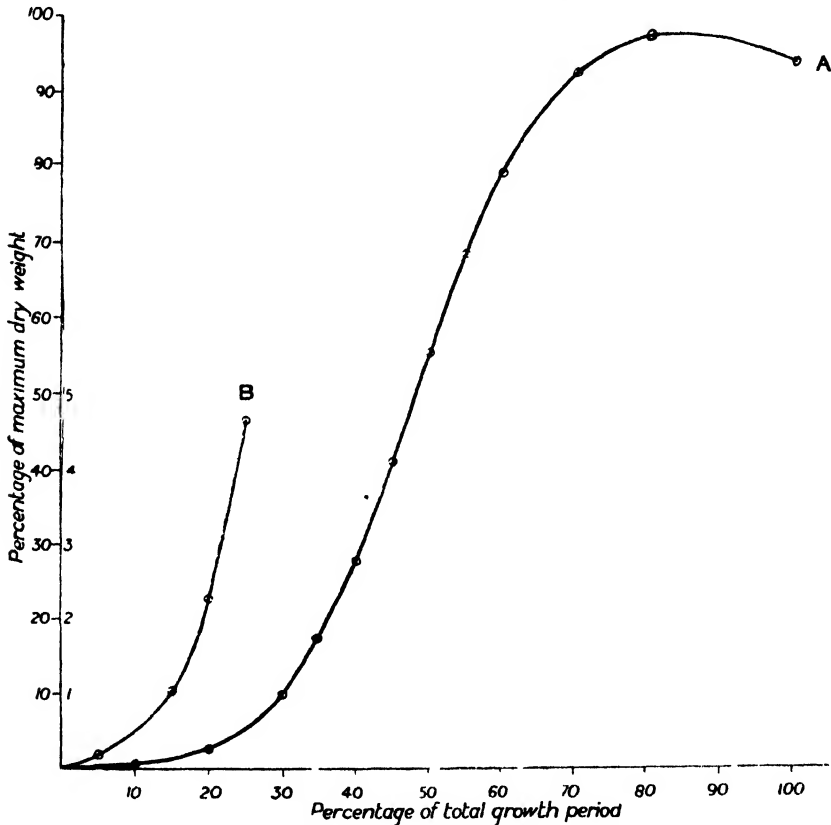


FIG. 3. A. Mean curve of total dry weight. B. First portion of curve A on larger scale.

increase, and the method of handling the problem in the two cases is identical, they will be discussed together here. The curve of dry weight increase is shown in Fig. 3.

If the environmental conditions remained constant at their average magnitudes, the shapes of the curves of leaf-area and dry weight would still remain of the same form. In other words, the succession of growth rates indicated by the changing slopes of the curves is determined largely by internal physiological factors. The effect of the environment is reflected in local changes in the slopes and by a shift in the points of inflexion and a change in the end points.

Under environmental conditions artificially maintained at a constant level the changing slope of the curve would be conditioned entirely by the changing internal factors of the plant. Such a curve might be called a 'normal curve'. The shape of the 'normal curve' will of course vary with the level at which the external factors are maintained; indeed this has been experimentally verified by the author for cucumbers growing under constant conditions of temperature and light.

If the shape of the 'normal curves', showing the changing rates of the two processes with time under constant average conditions, were known the effect of changes in the environment might be measured by comparing the slopes of comparable portions of the experimental and normal curves. This is essentially the method which has been utilized. The experimental curves for single experiments during the four years, however, differ among themselves in many respects. The period of the growth cycle varies from year to year, and the maximum points of the leaf-area and dry weight curves vary with the season and with the type of manuring used. All the curves have therefore been standardized by taking the period of the whole cycle as 100, and the maximum attained as 100, and re-calculating for each sample taken the time after germination as a percentage of the whole time, and the magnitudes of leaf-area and dry weight as percentages of the maximum value attained in the series to which they belonged. In this way the curves can all be presented on the same scale. Fig. 2 shows the two extreme curves for leaf-area derived from the experimental data of 1922 B and 1924 and treated in this way, as well as the normal (mean) curve obtained by averaging the values of single experiments.

In order to obtain an approximation to the 'normal curves', smooth curves were drawn through the experimental points representing each series, and the values of leaf-area and dry weight read off at comparable time intervals, viz. 10 per cent., 20 per cent., &c., of the whole period. Geometric means of the values so obtained were taken, and from these the mean curves (approximate 'normal curves') shown in Figs. 2 and 3 were constructed. Geometric means rather than arithmetic means were taken, as it was rates of growth which were to be averaged. The curves so obtained have not been smoothed, and show no marked irregularities.

These curves were treated as approximations to the normal curves required, and were taken to represent the succession of growth rates as determined by internal factors alone under a uniform environment. All that remained to be done was to re-calculate the value of each sample in all the series, taking the maximum value experimentally found and reading off the normal curve the percentage of this value corresponding with the percentage of the total time cycle which had elapsed when the particular sample in consideration was taken. In this way the experimental curves were restored to their original scale of time and magnitude and the correspond-

ing values of a similar normal curve were known. The napierian logarithms of each value for experimental and calculated normal series were taken, successive values for weekly samples in each case were subtracted, and thus the relative growth rates over comparable periods for experimental and normal curves were known. The differences between these relative rates measured the effects of changing environment. Thus, if the difference was zero, there was no effect, while acceleration and inhibition gave positive and negative values of the difference respectively. The process of calculation is shown for the leaf-area figures of 1921 in Table III. Only the portion of the curve previous to maximum leaf area was used, as already indicated. The whole time period, which was taken as 100, for relative leaf growth rate calculations was the number of days which elapsed from germination to the death of the leaves, but for efficiency index the whole life-cycle up to harvest was utilized.

TABLE III.

Days.	% Whole Period.	Actual Area.	Area calculated from Normal Curve.	Rel. Growth Rate.		Difference.
				Actual.	Calc. Normal.	
7	7.7 %	8.93 sq. cm.	89.9 sq. cm.			
14	15.4 %	28.9 "	32.4 "	1.1745	1.2820	-0.1075
21	23.1 %	90.3 "	91.2 "	1.1393	1.0350	+0.1043
28	30.8 %	190 "	229 "	0.7439	0.9206	-0.1767
35	38.5 %	479 "	521 "	0.9246	0.8221	+0.1025
42	46.2 %	787 "	832 "	0.4966	0.4680	-0.0289
49	53.8 %	899 "	881 "	0.0553	0.0573	-0.0018

The differences in the relative growth rates, such as are shown in the last column of Table III, were those utilized for calculating the correlation coefficients. The factors studied were :

1. Av. day temperature ( $T$ ).
2. Av. night temperature ( $t$ ).
3. Total radiation ( $L$ ).
4. Net assimilation rate ( $R_n$ ).
5. Relative leaf growth rate ( $R_r$ ).
6. Evaporating power of the air ( $E$ ).

The factors 1, 2, 3, and 5 were used for the first set of partial correlations, and the partial correlations are shown below, together with the regression equation :

$$r_{15.23} = +0.578, \text{ significance } > 100 : 1.$$

$$r_{28.13} = -0.474, \quad \text{,,} \quad 100 : 1.$$

$$r_{35.12} = -0.383, \quad \text{,,} \quad 21 : 1.$$

$$R_e = 0.1430 T - 0.1172 t - 0.2814 L - 1.3504. \dots (2).$$

As with net assimilation, the positive effect of day temperature and negative effect of night temperature appear, but a totally different effect of

radiation is seen. High radiation is *negatively* correlation with relative leaf growth, which indicates that, after allowance has been made for the high temperature associated with light sunshine, the effect of strong radiation on leaf growth is inhibitory. Whether this holds over the whole range of light intensities cannot be ascertained from these data, but it is quite clear that for leaf growth the average intensity of light during the summer is in excess of the needs of the plant. Confirmatory evidence of this for barley is seen in certain experiments of Dr. W. E. Brencley,<sup>1</sup> in which plants crowded together were compared with plants widely spaced. As each plant was grown singly in water culture there was no question of limited water or nutrients. The striking differences in leaf-area in the two sets are clearly seen in the published photographs. The conclusion that the effect was due to humidity differences is not conclusive; it may have been due to the reduction of light intensity in consequence of crowding leading to higher relative leaf growth rate in accord with the negative correlation here established. In view of the fact that in spite of a larger leaf surface the dry weights of the crowded plants fell much below that of the spaced, it is clear that the net assimilation rate fell with the low light intensity, as would be expected from the high positive correlation of net assimilation and light recorded above. An optimum for leaf growth is probable at low intensity of light; an investigation to test this possibility the author hopes shortly to undertake.

The magnitude of the negative correlation with night temperature is difficult to understand. It has been seen that radiation has an inhibitory effect on relative leaf growth, and hence it would be supposed that this process would be most active during the night. Whence, then, the negative correlation with night temperature? A possible explanation which presented itself was that the detrimental effect of high night temperature was bound up with the negative correlation of net assimilation rate with night temperature, and might be due to respiration losses. To test this hypothesis a new set of partial correlations are calculated, bringing in net assimilation rate as one of the variables.

The result is presented below :

1. Av. day temp.
2. Av. night temp.  $r_{15 \cdot 234} = +0.527.$   $r_{45 \cdot 123} = +0.087.$
3. Total radiation  $r_{25 \cdot 134} = -0.410.$
4. Net assimilation rate  $r_{35 \cdot 121} = -0.382.$
5. Rel. leaf growth rate

$$R_l = 0.1316T - 0.1085t - 0.3086L + 0.1524R_a - 1.4266. \dots (3).$$

The values of the new partial correlation coefficients have scarcely

<sup>1</sup> W. E. Brencley: Some Factors in Plant Competition. *Ann. App. Biol.*, vi, pp. 142-70, 1919.

changed from those given above, and the surprising result emerges that the correlation between net assimilation and relative leaf growth, although positive, is almost negligible. The experiments on competition by Dr. Brenchley, already quoted, seem to bear out this conclusion, for there large leaf surface is associated with low net assimilation, and vice versa. Bearing in mind that zero correlation means a 'normally' maintained growth rate, it is clear that leaf growth is scarcely at all affected by variations in net assimilation rate. This seems to indicate that under weather conditions such as prevail during early summer in this country net assimilation is maintained at such a level that the carbohydrate material formed is always in excess of the immediate demands of the plant for leaf growth material, and excess must be laid down as reserve. It rarely happens apparently that adverse weather conditions last long enough to exhaust these reserves of material for maintaining leaf growth.

Strong evidence for the independence of relative leaf growth rate and net assimilation has also been found by the author in experiments with cucumbers at high temperatures in artificial light, where rapidly falling relative leaf growth rates have been found to accompany steady assimilation rates.

Dr. W. Brenchley's experiments seem also to point in this direction, and it is to be regretted that data of leaf-area were not then taken, as with such additional information the problem of the relation of leaf growth and assimilation rate in her experiment could have been elucidated.

The cause of the harmful effect of high night temperatures on leaf growth must be sought elsewhere. The evidence seems to point to a beneficial effect on the processes of leaf growth and assimilation of large fluctuations, as such, of temperature between day and night, though what this means cannot be formulated in precise terms.

The relative leaf growth rates, calculated from the regression equation (2), together with the values calculated from experimental data, are given in Fig. 4. The agreement in general is good, but is not so satisfactory as that for net assimilation. It must be remembered that, firstly, the 'normal curve' of leaf growth is only approximately known, and, secondly, that sampling errors will play a very large part in determining the results, since we are dealing with differences in slope at points on a curve, and indeed differences between differences.

The correlation coefficients are sufficiently significant when the small number of experimental values is taken into account. Moreover, although the differences in manuring can have only a small effect on the shape of the normal curve, it is unlikely that no influence at all is exerted. Rippel and Ludwig<sup>1</sup> indeed claim to have demonstrated such a change in shape for the dry weight increase curve.

<sup>1</sup> A. Rippel and O. Ludwig : Untersuchungen über physiologische Gleichgewichtszustände bei Pflanzen, &c. Biochem. Zeitschr., 1925.

The consistent variation in level of the calculated and experimental values in the curves for 1923 and 1924, as seen in Fig. 4, do in fact point to such an effect. It will be seen, however, that the changes in direction of the curves follow each other closely, indicating that the correlation coefficients are correct in sign, and substantially so in magnitude.

Comparing the curves for experimental values in Figs. 1 and 4, the similarity in form is striking. This similarity is confirmed by taking the correlation coefficient of the differences of assimilation and leaf growth rates from their means. This is found to be + 0.556. If this were the sole information on the subject the conclusion that leaf growth and assimilation are highly correlated would be irresistible. The association has been shown to be spurious, and is due to the fact that both processes are highly correlated positively with day temperature.

In order to test whether humidity plays an important part, as Dr. W. Brechley has suggested, a third set of partial correlations was calculated, in which evaporating power of the air replaced net assimilation rate, which was known to have little effect. The values are as follows :

- |                                 |  |
|---------------------------------|--|
| 1. Av. day temperature          | $r_{15 \cdot 230} = +0.487$ , significance > 100 : 1 |
| 2. Av. night temperature        | $r_{25 \cdot 136} = -0.391$ , „ 24 : 1               |
| 3. Total radiation              | $r_{35 \cdot 236} = -0.422$ , „ 37 : 1               |
| 5. Relative leaf growth rate    | $r_{56 \cdot 12,1} = +0.228$ , not significant.      |
| 6. Evaporating power of the air |  |

Again the correlations previously established remain almost unchanged, and it is seen that the partial correlation with evaporating power is positive and fairly large, but not significant. This was quite unanticipated, as it was expected that high humidity would favour leaf growth. Attention has already been called to the unsatisfactory nature of the evaporation data, but as they were the only ones available they were perforce used. The effect found may possibly be due to rainfall causing water-logging of the soil. During rainy weather continual saturation of the soil, by checking aeration, may have acted adversely on leaf growth, so that the positive correlation may mean the beneficial effects of lower water-content of the soil. However this may be, undoubtedly the rate of uptake of nitrogen will influence leaf growth rate, and this factor has not been taken into account. Heavy rainfall will tend to wash out the nitrate, which would account in part for the adverse effect found to be associated with high humidity of the air, and as the drainage water from the pots was replaced as soon as the weather cleared, the positive correlation with low humidity would tend to be favoured by this also. (In this connexion see Addendum.)

# on the Growth of Barley.

19

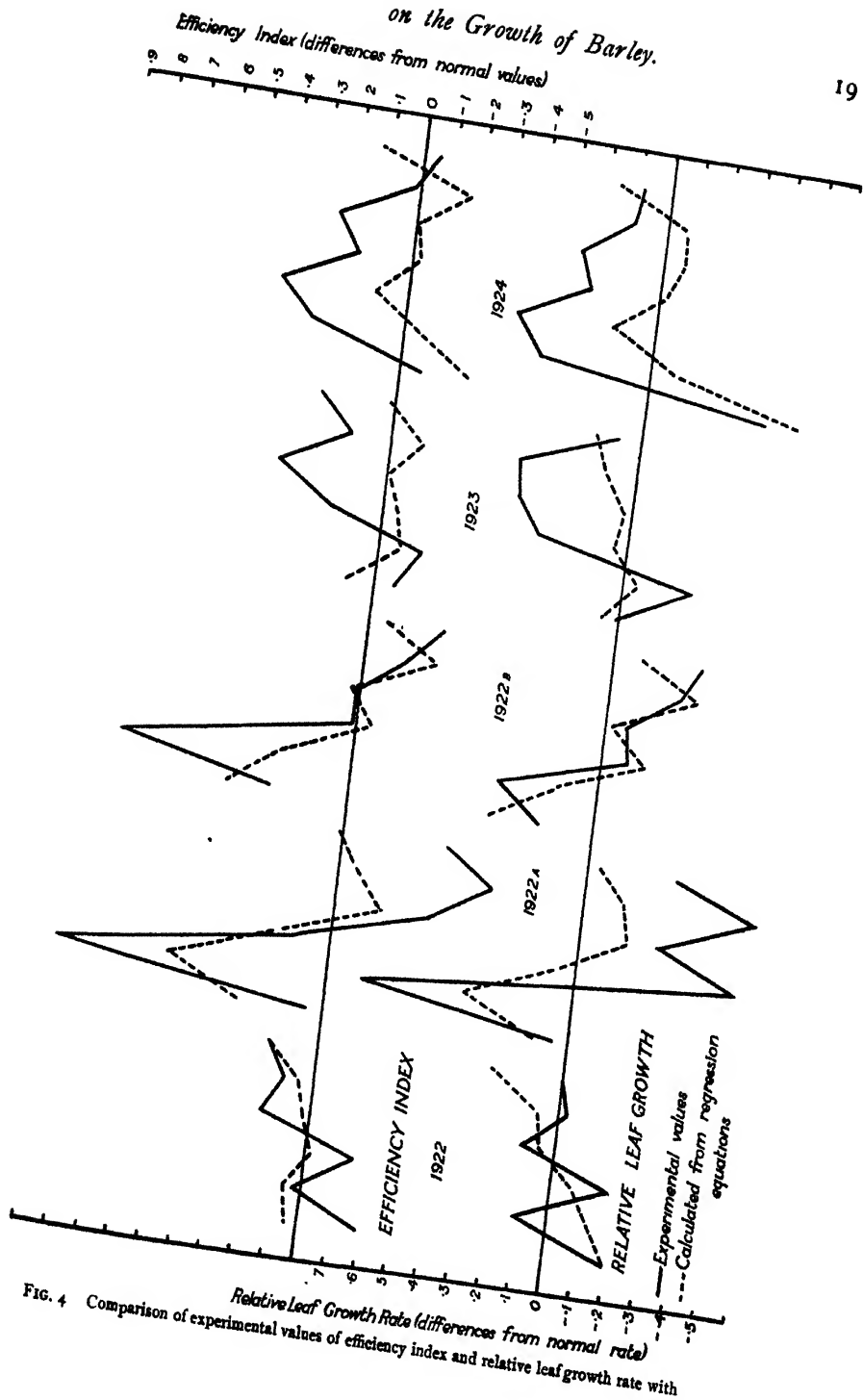


FIG. 4 Comparison of experimental values of efficiency index and relative leaf growth rate with



Reviewing the information thus far obtained, a clear picture of the adaptation of the barley plant to climatic conditions emerges. The positive and negative correlations of net assimilation and relative leaf growth rates respectively with total radiation provide a mechanism whereby the yield of the plant tends to remain within fairly narrow limits, in spite of climatic variation. Yield depends on total material assimilated, which in turn is determined by two processes, which may by analogy be termed intensity and capacity factors of total assimilation.

The intensity factor is the net assimilation rate which determines the effectiveness with which the leaf surface provides material, while the leaf surface growth, as capacity factor, limits the size of effective area. In a season of high total radiation the negative correlation will condition a low level of leaf growth rate, associated with a high net assimilation, while in a wet season the reverse relations will hold. The product of the two processes will maintain the total of material assimilated within a fairly narrow range. It is, however, necessary to bear in mind that the high temperatures prevailing during bright weather will increase the rate of nitrification in the soil and thus indirectly lead to greater leaf growth by increase of available nitrogen.

#### *The Effect of Climatic Conditions on Efficiency Index.*

The relative rate of dry weight increase is quite analogous with relative leaf growth rate, and may be represented as

$$R_w = \frac{1}{w} \cdot \frac{dw}{dt}.$$

This is the same function as the Efficiency Index of Blackman,<sup>1</sup> and the mean rate is calculated by subtracting the natural logarithms of the weights at beginning and end of a unit time-period.

For the purposes of correlation the whole growth cycle was divided into two parts at the point corresponding with maximum leaf-area.

The two parts were dealt with in different ways, the reason for which will be made clear later.

The 'normal curve' of dry weight increase was obtained by taking geometric means of corresponding values on the standardized experimental curves in a manner similar to that by which the normal leaf-area curve was calculated. The normal curve of dry weight increase is shown in Fig. 3. The early part of the curve is shown separately on a larger scale. The

<sup>1</sup> V. H. Blackman : The Compound Interest Law and Plant Growth. *Ann. Bot.*, xxxiii. 353, 1919.

comparison of experimental efficiency indices and the corresponding 'normal rates' are shown for 1921 in Fig. 5.

The shape of the normal curve of efficiency index need not be discussed here, but it is clear that the growth rate as indicated by the changing slope of the curve is no simple function of time.

If it could be shown that the weight at each moment were related

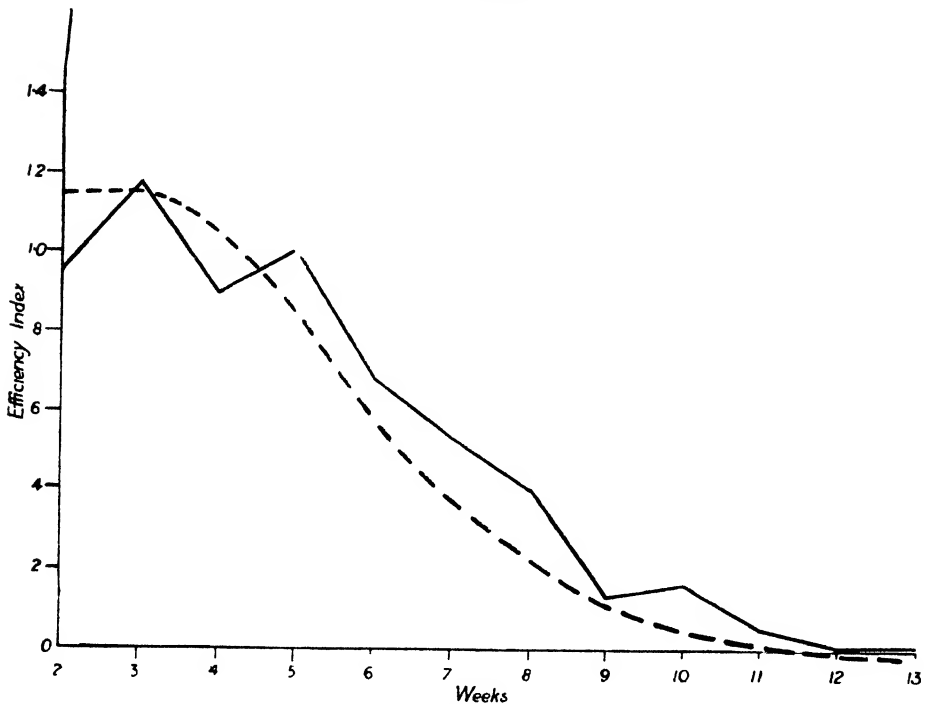


FIG. 5.

in a simple manner to the efficiency index, time as a variable could be eliminated and in its stead the actual weight could be used, and the variation in efficiency index could be stated in terms of percentage of final weight attained. This method of dealing with the problem was suggested to the author by Mr. R. A. Fisher, of Rothamsted Experimental Station. Fig. 6 shows the efficiency index plotted against the percentage of final weight for the series of values of the 'normal curve' (Fig. 3).

It is clear that after 40 per cent. of the final weight has been reached the relation between the variables is strictly linear. The 40 per cent. points corresponds in fact with the point of maximum tillering, and is a critical point in the life-cycle. It is interesting to note that the linear relation of efficiency index and absolute weight is the crucial test for the auto-catalytic nature of the growth process, and Fig. 6 abundantly demonstrates

that only the latter part of the growth cycle can be represented by an autocatalytic reaction equation.

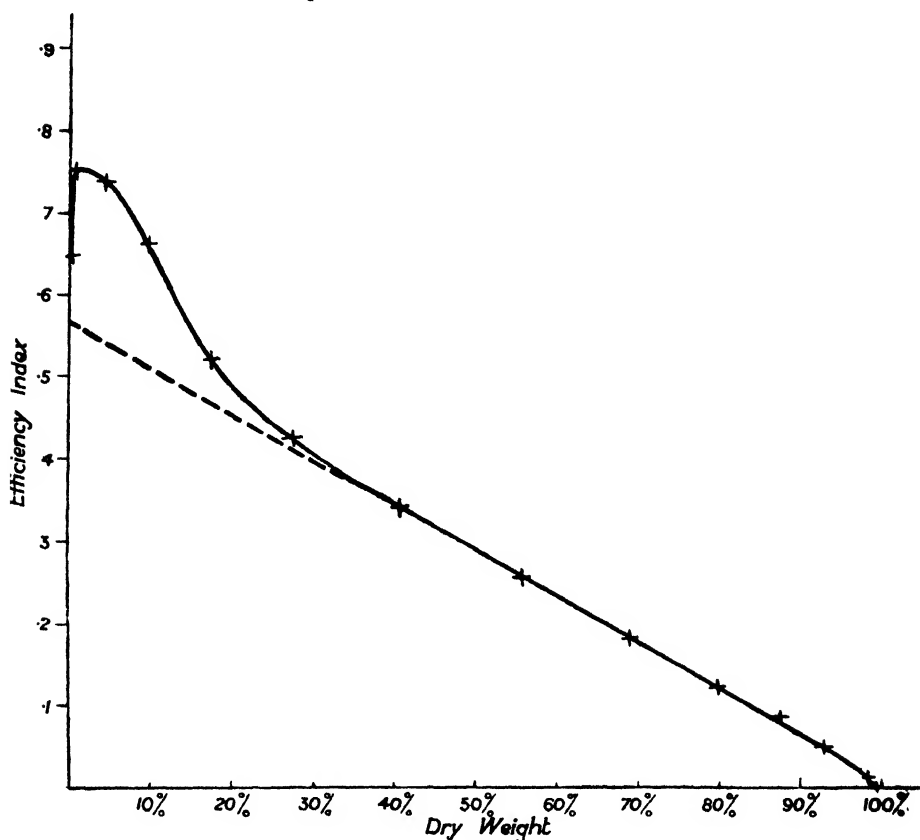


FIG. 6.

*Correlations of Efficiency Index with Climatic Factors in First Part of Growth Cycle.*

The method used was similar to that previously described for relative leaf growth. The factors included were :

1. Average day temperature ( $T$ ).
2. Average night temperature ( $t$ ).
3. Total radiation ( $L$ ).
7. Efficiency index.

The partial correlations and regression equation are presented below:

$$r_{17.23} = +0.441, \text{ significance } 50:1$$

$$r_{27.13} = -0.389, \text{ " } 25:1$$

$$r_{37.12} = -0.073, \text{ not significant.}$$

$$R_w = 0.1075 T - 0.0999 t - 0.0541 L - 0.797.$$

The only point calling for comment is the partial correlation with total radiation, which is almost negligibly small. This indicates that efficiency index is almost independent of radiation, and that the inverse relations of net assimilation and relative leaf growth with radiation almost exactly balance one another, so that increase in dry weight proceeds at its 'normal rate' whatever the conditions of illumination may be. This, of course, holds true only for the range actually explored, namely, average early summer conditions. The relations of net assimilation and relative leaf growth rate with efficiency index are given by the two following correlation coefficients:

- |                              |                               |
|------------------------------|-------------------------------|
|                              | 1. Av. day temp.              |
|                              | 2. Av. night temp.            |
| $r_{47 \cdot 1235} = +0.646$ | 3. Total radiation.           |
| $r_{57 \cdot 1234} = +0.804$ | 4. Net assimilation rate.     |
|                              | 5. Relative leaf growth rate. |
|                              | 7. Efficiency index.          |

—which show that after allowance has been made for the effect of external factors, efficiency index is strongly correlated with net assimilation and relative leaf growth, and, furthermore, the latter is predominant in effect.

The small correlation of efficiency index with hours bright sunshine found by Dr. W. Brenchley for peas in the early stages of growth ( $-0.0132$ ) may possibly be explained in the same way as the small correlation here found with total radiation. Intense light may in this case also have an inimical effect on relative leaf growth, but the later positive correlation indicates that the detrimental effect is later replaced by a beneficial one. Further investigation of this point is urgently needed, as in this way only can the 'light requirements' of plants be interpreted.

### *Second Part of Growth Cycle.*

The factors included in the analysis are:

1. Average day temperature ( $T$ ).
2. Average night temperature ( $t$ ).
3. Total radiation ( $L$ ).
5. Efficiency index ( $R_w$ ).
8. Dry weight ( $W$ ).

$$\begin{aligned}
 r_{15 \cdot 238} &= +0.388. \\
 r_{25 \cdot 138} &= -0.292. \\
 r_{35 \cdot 128} &= -0.266. \\
 r_{85 \cdot 127} &= -0.942.
 \end{aligned}$$

The linear relation between dry weight and efficiency index is reflected in the very high value of the partial correlation  $r_{85 \cdot 127}$ . The negative sign

indicates the fall in efficiency index as growth approaches its completion. As the ripening process advances assimilation gradually ceases, and this is responsible for the smaller values of the correlations with temperature. The adverse effect of high night temperatures is still apparent. The negative correlation with total radiation has increased considerably, and is easily explained by the observed effect of high light intensity in hastening the dying off of the leaf surface.

The experimental values and those calculated from the regression equation for the first part of the growth cycle are shown in Fig. 4. The general similarity of the curve with the other two already considered is clear, and is due to the high correlation of efficiency index with the other two processes.

From the facts emerging in this analysis the interrelations of the processes determining growth are shown, and the interaction of the climatic complex with the internal factors. This analysis is in the nature of a preliminary survey of the physiological aspects of the problem of the adaptation of the plant to the environment. As suggested in the introductory remarks, the way is indicated towards a true agricultural physiology, which may restate in precise terms much that at present is empirical knowledge. The study of the plant as a whole is needed to test conclusions drawn from laboratory experimentation with single organs where previous history has been assumed to be unimportant, and on single phases of the total life-cycle, whose chief characteristic is an intrinsic unity.

In conclusion, the author wishes to thank Sir John Russell for facilities for experimental work which was carried out at the Rothamsted Experimental Station, Prof. V. H. Blackman for continued interest and inspiration, Mr. R. A. Fisher for invaluable suggestions and patient criticism, and lastly Miss E. D. Kay, without whose co-operation and unflagging industry the necessary routine work of amassing data could not have been completed.

#### SUMMARY.

An analysis of the effect of seven environmental factors on the growth of barley in pot culture has been undertaken.

The environmental factors are :

1. Maximum day temperature.
2. Average day temperature.
3. Minimum night temperature.
4. Average night temperature.
5. Total radiation in calories per sq. cm. per week.
6. Hours bright sunshine.
7. Evaporating power of the air.

Three measures of growth are dealt with :

1. Net assimilation rate (dry weight increase per unit leaf-area per unit time).
2. Relative rate of growth of leaf surface.
3. Relative rate of increase in dry weight (efficiency index).

The whole growth cycle is divided into two parts at the point at which maximum leaf-area occurred, and each half is considered separately.

*Net assimilation rate* during the first part of the growth cycle is shown to be independent of time and of the quantity of nitrogen added as manure. The partial correlations of assimilation rate with day temperature and radiation are significantly positive and high in value, while a significant negative correlation with night temperature obtains. The values of net assimilation rates calculated from the regression equation are graphically presented together with the experimental values, and good agreement is found. Over 80 per cent. of the variation in assimilation rate is accounted for by variation in external factors.

*Relative leaf growth rate and efficiency index.* The method used for the study of the variations in these processes due to environmental changes consists essentially in determining the approximate form of 'normal curves' for the two cases, such that varying rates are conditioned solely by internal factors, while the external factors may be supposed to remain constant at their mean values.

The differences in the slopes of comparable portions of the experimental and 'normal curves' are taken to represent the effects of the external factors, an increase in slope indicating acceleration, and a decrease inhibition. Partial correlations of relative leaf growth rate and average day temperature are significantly positive, and a significant negative correlation with night temperature is again found. A large significant negative correlation with total radiation appears, while relative leaf growth is found to be almost independent of net assimilation rate. A spurious correlation between net assimilation and relative leaf growth is due to both processes being highly positively correlated with average day temperature and negatively with average night temperature.

*Efficiency index* in the first part of the growth cycle is almost independent of radiation, but is highly positively correlated with average day temperature and significantly negatively with night temperature. In the second part of the growth cycle all the correlations decrease in magnitude with the onset of ripening.

The physiological adaptation of the barley plant to climatic changes is brought out clearly by the partial correlations. The antagonistic effects of the correlations with total radiation, *positive* in the case of net assimilation, *negative* in the case of relative leaf growth rate, tend to maintain the yield

constant. In absence of bright sunshine, in so far as the temperature does not fall, the high relative leaf growth rate will lead to a large leaf surface, and hence compensate for the low net assimilation rate, and vice versa. This compensating effect, however, is partially masked by the high nitrification rate in the soil associated with high soil temperature and hence with total radiation; high nitrogen content in the soil will tend to increase leaf growth irrespective of the inhibiting effect of bright sunshine.

#### *Addendum.*

Since the foregoing was written the analysis has been carried a step farther, and the influence of the nitrogen factor in growth has been investigated. It has been shown that the discrepancies in relative leaf growth rate between the experimental values and those calculated from the regression equation (shown graphically in Fig. 4) can largely be accounted for by the nitrogen relations. The residual values for relative leaf growth rate, namely, the differences unaccounted for by the variations in external factors, have been correlated with the departures from the mean values at the times of sampling of the nitrogen content of the leaves expressed as percentage nitrogen in the dry weight. The correlation coefficient is  $+0.635$  (significance  $> 100:1$ ). It thus appears that the consistent difference of level between experimental and calculated values for 1923 and 1924 is due to a consistent high value, during these two periods, of the nitrogen content of the leaves. As this figure for nitrogen content is independent of leaf-area there can be no question here of a spurious correlation arising from an indirect correction for leaf-area discrepancy.

The correlation between the same residuals and the ratio of weight of nitrogen in the leaves to the weight of nitrogen in the remainder of the plant is found to be  $+0.632$ , which is almost identical with the correlation value already given. As here the leaf-area is indirectly involved, since the total weight of nitrogen in the leaves depends on the total leaf surface, this correlation standing alone might be attributed to a spurious relation. In view, however, of the identity of the two values it seems probable that the excess nitrogen content of the leaf is accompanied by a corresponding high nitrogen content of the rest of the plant.

Evidently the discrepancies observed in Fig. 4 are not fortuitous, but indicate that relative leaf growth rate is largely dependent on internal factors and is relatively independent of external conditions; whereas, in contrast with this, net assimilation rate has been shown to be wholly controlled by external factors.

# **A Physiological Study of Varietal Differences in Plants.**

## **Part I. A Study of the Comparative Yields of Barley Varieties with Different Manurings.**

BY

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AND

FRANK CROWTHER, B.Sc.<sup>1</sup>

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WITH AN APPENDIX

BY

E. S. BEAVEN, Hon. LL.D. (Cantab.).

### **INTRODUCTION.**

**I**T is well known that varieties of any one plant frequently differ markedly in their yielding capacity. These differences presumably arise from differences in the absorption and efficient utilization of soil constituents. This paper describes an investigation undertaken to determine whether the *comparative* yields of varieties are independent of the total and relative amounts of fertilizers added, or whether individual varieties respond to varying extents to fertilizers.

Previous work on the physiological differences of varieties has been confined chiefly to a study of the amounts of manurial constituents removed by similar varieties and to differences in the resistance of varieties to toxic substances. Brown and MacIntire (2) showed that varieties of wheat differed in the amounts of nitrogen, phosphate, and potash absorbed during early growth. Gericke (5) found that the effect of fertilizer treatment at specific stages of growth differed with different varieties of wheat. The only investigation concerned with the effect of manuring on the comparative yield of varieties appears to be that of Fisher and Mackenzie (4), who, whilst dealing with the statistical treatment of crop variation, did not

<sup>1</sup> Ministry of Agriculture Research Scholar.



obtain a significant value for the differential effect of manuring on the yields of varieties of potatoes grown in the field. Beaven (1) described experiments with barley varieties grown under various manurings and obtained indications of a differential response of the varieties to manuring.

#### EXPERIMENTAL METHOD.

In order to make the investigation a comprehensive one a wide range of manuring was employed, with especial reference to the increasing deficiency of each important constituent separately. Such a range of manuring of necessity resulted in a wide range of yields, and the varietal differences tended to be outweighed by the much larger differences due to the manuring. It was expected from the negative result obtained by Fisher and Mackenzie that the effects of a differential varietal response would be of small magnitude, and would require data of a high order of precision for their establishment. Previous experience with pot-cultures in white quartz sand showed that this method was capable of high precision, and this technique seemed well adapted for the investigation of the problem in question. Further, the method facilitates the taking of measurements on the plants, and it was possible to control more readily than in soil-cultures the amounts of nutrients supplied to each plant.

Five well-known pure line varieties of two-rowed barley were used, viz.: Goldthorpe (G.), Plumage (P.), English Archer (E.A.), Plumage-Archer (P.-A.), and Spratt-Archer (S.-A.).<sup>1</sup>

As the experiment involved the use of 385 pots the plants were of necessity grown in the open; watering, which was rarely necessary, was carried out with tap-water at air temperature. Waterlogging in the pots was avoided by allowing percolation, and collecting the percolated water in bottles and subsequently returning it, as described by Gregory (6). The water content could not be controlled by periodic weighings owing to the size of the experiment. Complete randomizing of the pots was not possible owing to the complications in manuring and of taking measurements throughout the season; place-effect, however, would seem to be negligible in pots grown in the open. White glazed pots, 10 in. by 10 in., were employed, holding 30 lb. of dry sand. The manuring scheme employed is given in Table I.

The complete manure provided nitrogen (N), potash ( $K_2O$ ), and phosphoric acid ( $P_2O_5$ ) in the ratio of 3:2:1. In each 'deficiency series' the two manurial constituents not in deficiency were supplied in the same concentration as in the series with complete manure, while the third was present in 0, 1/25th, and 1/5th of the complete amounts. This gave

<sup>1</sup> Grain of the last four varieties was obtained from Dr. E. S. Beaven, to whom the authors wish to express their gratitude.

a range of grades of deficiency in one constituent in the presence of adequate amounts of the others. The 'complete manure' type was duplicated; thus eleven series in all were employed. For each manurial type the five varieties were replicated seven times, making a total of 385 pots used in the experiment. The manures were added in solution: nitrogen was added as  $\text{NaNO}_3$ , phosphate as  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ , and the potash as  $\text{K}_2\text{SO}_4$ . In this way no two of the manurial constituents studied were added as a single salt. The resulting variation in the amounts of sodium was

TABLE I.

*Manuring Scheme (Amounts in Grammes per Pot).*

	<i>Phosphate-deficient.</i>			<i>Complete.</i>	<i>Nitrogen-deficient.</i>			<i>Complete.</i>	<i>Potash-deficient.</i>		
	<i>A.</i>	<i>B.</i>	<i>C.</i>	<i>D.</i>	<i>E.</i>	<i>F.</i>	<i>G.</i>	<i>H.</i>	<i>I.</i>	<i>J.</i>	<i>K.</i>
$\text{P}_2\text{O}_5$	0	0.02	0.10	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
N	1.50	1.50	1.50	1.50	0	0.06	0.30	1.50	1.50	1.50	1.50
$\text{K}_2\text{O}$	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0	0.04	0.20

corrected by adding  $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$  where necessary. On the basis of a 20 per cent. water content of the sand, the sodium concentration was 0.1 per cent., and the whole salt concentration 0.5 per cent., of the total solution. The concentration of sodium was far below that necessary to produce the slightest toxic effect as determined by Lipman, Davis, and West (8) working with barley and sodium chloride. The only ion other than those under consideration which varied was ( $\text{SO}_4$ ), which has been shown to have a very small effect on growth (7). The solutions were brought to an initial pH 6.8 by adding sulphuric acid. Calcium (0.37 grm.  $\text{CaCl}_2$ ), magnesium (1.25 grm.  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ), and a trace of iron were added in like amounts to all pots. All the manures were added before sowing.

The seed used was selected for uniformity in size and colour by eye and nine seeds sown per pot. Experience has shown that a more uniform set is obtained in this way than by selecting the seed by weight and sowing fewer seeds per pot. It was impossible to avoid using two-year-old seed for Goldthorpe; this could not influence the differential effect to be studied. The pots were sown on May 2, 1927, and germination was complete by May 11. A fortnight after germination the plants were reduced to four per pot by removing those of extreme sizes. The final reduction to three per pot was made after leaf measurements had been taken on all the plants, and those retained which left a total leaf-area per pot closely approaching the average for the group of replicates.

Leaf measurements were taken on the plants in the types A, E, and I, i. e. those completely deficient in one constituent until the time of maximum

leaf-area. Tiller counts were taken weekly throughout the season on all pots. At the period of approximately maximum leaf-area (mid-July) a sample consisting of fifty-five pots, one of each of the replicates, was taken, and planimeter measurements of leaf-area, together with dry-weight data for stem, leaves, and roots, were obtained.

The time of ripening extended from Sept. 8 to Oct. 14. This enabled each group of replicates of any one variety and manuring to be harvested when ripe, without reference to the ripeness of the other varieties in the same or in other manurings. Tiller-length measurements with numbers of sterile and fertile grains, and dry-weight data for ears, straw, and roots, and corn after threshing, were obtained on the nitrogen- and phosphate-deficient series. The potash-deficient series produced plants which could not be treated in this manner. The plants in types I and J produced abundant tillers, short stems, and only rarely an ear, which was green and sterile: they were harvested whilst still green in mid-October, by which time some plants were already dead. The type K behaved similarly to the 'complete manure' plants until mid-August, when the straw collapsed suddenly; the grain was allowed to become fully ripe before harvesting.

Ten pots had to be discarded owing to death of the plants from wire-worm or injury by *Helminthosporium*.

The present paper deals only with the final dry-weight data.

TABLE II.  
*Dry Weights (Grammes per Pot).*

EARS—		Variety.				
	Manuring.	G.	P.	E.A.	P.-A.	S.-A.
P <sub>2</sub> O <sub>5</sub> deficient	A	1.52	2.14	2.18	1.78	2.14
	B	2.93	4.57	4.35	2.77	3.94
	C	12.64	13.09	19.17	15.69	12.74
Complete	D	44.01	51.65	55.63	59.34	51.79
N deficient	E	0.63	1.00	1.18	0.94	1.17
	F	2.08	2.21	3.31	2.52	2.35
	G	8.11	13.37	14.83	15.47	12.41
Complete	H	41.66	51.50	58.67	56.88	54.00
K <sub>2</sub> O deficient	I	0	0	0.78	0	0.22
	J	0	0.54	0.76	0	0
	K	22.32	20.19	19.71	23.88	19.11

TABLE II (*continued*).

*STRAW—*

		<i>Variety.</i>				
	<i>Manuring.</i>	<i>G.</i>	<i>P.</i>	<i>E.A.</i>	<i>I.-A.</i>	<i>S.-A.</i>
$P_2O_5$ deficient	A	11.24	11.72	13.39	11.50	9.87
	B	15.31	17.03	16.77	13.63	14.57
	C	33.49	35.66	38.00	33.30	32.89
Complete	D	69.14	78.89	67.98	68.83	69.67
N deficient	E	1.99	1.93	2.27	2.30	2.72
	F	5.61	4.97	5.83	6.92	5.47
	G	18.85	21.97	22.48	24.03	21.62
Complete	H	67.84	79.94	70.36	68.22	72.97
$K_2O$ deficient	I	6.98	4.34	7.80	6.07	4.43
	J	12.09	9.21	14.02	12.09	11.20
	K	41.62	34.68	33.44	34.09	35.75

*ROOTS—*

$P_2O_5$ deficient	A	3.32	3.24	3.65	2.83	3.01
	B	5.21	4.40	3.94	2.73	4.25
	C	6.19	5.06	7.13	5.53	4.73
Complete	D	15.03	8.01	10.03	9.25	10.80
N deficient	E	0.70	0.67	0.97	0.76	1.07
	F	1.94	1.56	2.82	2.58	2.02
	G	3.53	3.45	4.25	5.92	5.12
Complete	H	15.15	9.77	9.39	9.61	15.71*
$K_2O$ deficient	I	1.16	0.64	0.94	0.81	0.56
	J	2.54	2.49	1.65	1.46	1.81
	K	8.13	6.86	4.12	6.80	8.08

\* Sample not washed free from sand.

ANALYSIS OF DATA.

Table II gives the yield for the five varieties in each of the manurial types, in grammes dry weight per pot, for ears, straw, and roots respectively. Each value is the mean of the replicates.

The data show that in each manurial type the varieties differ in yield, and that these differences are relatively greater in the deficient series than

in the complete manure. Also the relative positions of the varieties when arranged in order of yields vary with the manuring. To obtain information as to the significance of these differences the data were treated by the 'Analysis of Variance' method and 'Z' test (Fisher (8)). It will be convenient to discuss separately (a) a comparison of the varieties within each manurial type, and (b) a comparison of the varieties with change of manuring by treating together groups of manurial types.

(a) *Comparison of Varieties within Individual Types of Manuring.*

The horizontal rows of mean yields given in Table II were treated as separate experiments, each consisting of thirty pots forming a unit with the same manurial treatment, but with five varietal groups each of six replicates of the same variety. An estimate of the significance of the varietal differences in yield was obtained by a comparison of the variance of the five mean yields of the five varieties with the sum of the variances for the the groups of replicates of each variety. As an example the calculations for straw, manuring 'E', are given below :

	<i>Sum of Squares of Deviations.</i>	<i>Degrees of Freedom.</i>	<i>Mean Square.</i>	<i>Log<sub>e</sub> Mean Square.</i>	<i>'Z.'</i>
Within Varieties	3.0538	25	0.1222	3.89786	0.7875
Between Varieties	2.3610	4	0.5903	1.47286	

The deviations for 'Within Varieties' were measured from the mean of the replicates for each variety, the sum of the squares of the deviations being added for the five varieties. This grouping reduced the degrees of freedom available for the thirty pots to twenty-five. The deviations of the varietal means for 'Between Varieties' were measured from the mean of the varietal means of the five varieties: six times the sum of the squares of deviations was taken, since the variance was calculated on a basis of single pots. Where the replicates had been reduced to five owing to disease, the degrees of freedom available were correspondingly reduced. Table III gives the values obtained for 'Z' in the comparison of the variances for each part of the plant separately and in total.

The 5 per cent. probability value (the value at which the frequency of occurrence, owing to chance, of effects of this magnitude is only one in twenty times) is taken as the limit of significance. The 5 per cent. probability value of 'Z' for the degrees of freedom available ( $n_1 = 4$ ;  $n_2 = 24$ ) is 0.5106. All values above this are taken as significant, and are shown in heavy type in Table III.

Table III clearly establishes the significance of the observed differences between varietal yields in a majority of the manurial types. It should be noted that prevailingly higher values of 'Z' are obtained for ear than for

straw weights. This is surprising in view of the much greater variability in the former. The absence of significant effects in the I and J series is due to much greater variability of the replicates. That this was a direct result of the manuring was confirmed by an independent experiment, run concurrently, on the effect of different doses of potash, the same great variability being obtained in the potash-deficient pots.

TABLE III.

*Values of 'Z' for Significance of Differences between Varieties in each Manurial Type. (5 per cent. prob. value of 'Z' = 0.5106.)*

<i>Manuring.</i>	<i>Ears.</i>	<i>Straw.</i>	<i>Roots.</i>	<i>Total Tops</i>	<i>Grand Total.</i>
A	0.0948	0.6000	-0.1800	0.4132	0.3144
B	0.6413	0.4492	0.5636	0.5624	0.6201
C	0.8095	0.3542	0.5697	0.7046	0.7658
D	0.5379	0.2978	0.6145	0.0473	0.5679
E	0.6546	0.7875	0.1249	0.7560	0.6009
F	0.5731	0.8876	0.4989	0.7123	0.7162
G	1.3222	0.9757	0.5609	1.3617	1.2994
H	0.7104	0.2813	0.2323	0.2950	0.0730
I	—	0.0284	0.3700	0.0352	0.0705
J	—	-0.1393	0.3216	-0.1353	-0.4118
K	0.3690	1.0613	0.6910	0.7232	0.7836

The Mean Square value for 'Within Varieties' gave an estimate of the standard error of the replicates, since the variance of the mean is a sixth of the mean square value in each manurial type (a weighted mean was used when the replicates were reduced to five owing to disease). In this way one value of the standard error of the replicates was obtained from the thirty pots, there being no indication of prevailing greater variation in one variety than in another.

The statistical treatment has not given any indications as to which varieties in particular were responsible for the significant varietal responses, the standard error value being too high for obtaining significant differences on comparing the varieties in pairs. However, indications as to which varieties showed large differences in response can be obtained from the original data of mean yield.

The accompanying Table IV shows the results from comparisons of the varieties in pairs, for dry weights of ears, straw, and roots respectively. A difference of twice the standard error ( $\sigma$ ) in each manurial type was arbitrarily taken as a basis for comparison. In the table referred to, a + sign indicates

that the first variety in a comparison exceeds the second variety in yield by more than  $2\sigma$ , a — sign indicates that the second variety exceeds the first by more than  $2\sigma$ , whilst a o indicates that the difference between the varieties is less than  $2\sigma$ . It cannot be stated that all the differences exceeding  $2\sigma$  in Table IV are statistically significant, but the significant values of 'Z' shown in Table III for a particular manurial type are likely to be due to

TABLE IV.  
*Comparison of Varieties.*

EARS	(Differences more than 2 σ.)									Complete
	<i>P<sub>2</sub>O<sub>5</sub> deficient.</i>			<i>N deficient.</i>			<i>K<sub>2</sub>O deficient.</i>			
Varieties.	A.	B.	C.	E	F.	G.	I.	J	K.	D and H.
P.-G.	+	+	o	+	o	+			o	+
E.A.-G.	+	+	+	+	+	+			o	+
P.-A.-G.	o	o	+	+	o	+			o	+
S.-A.-G.	+	+	o	+	o	+			—	+
E.A.-P.	o	o	+	o	+	o			o	+
P.-A.-P	o	—	+	o	o	+			+	o
S.-A.-P.	o	o	o	o	o	o			o	o
P.-A.-E.A.	o	—	—	—	—	o			+	o
S.-A.-E.A.	o	o	—	o	—	—			o	o
S.-A.-P.-A.	o	+	—	+	o	—			—	o
STRAW—										
P.-G.	o	o	o	o	—	+	o	o	—	+
E.A.-G.	+	o	+	o	o	+	o	o	—	o
P.-A.-G.	o	o	o	+	+	+	o	o	—	o
S.-A.-G.	o	o	o	+	o	+	o	o	—	o
E.A.-P.	+	o	o	+	+	o	+	+	o	—
P.-A.-P.	o	—	o	+	+	+	o	o	o	—
S.-A.-P.	—	—	o	+	o	o	o	o	o	—
P.-A.-E.A.	—	—	—	o	+	+	o	o	o	o
S.-A.-E.A	—	—	—	+	o	o	—	o	+	o
S.-A.-P.-A.	—	o	o	+	—	—	o	o	o	o

+ indicates first variety exceeds second by  $2\sigma$ .

— „ second „ „ first by  $2\sigma$ .

o „ varieties do not differ by  $2\sigma$ .

TABLE IV (continued).

(Differences more than  $2\sigma$ .)

ROOTS—

Varieties.	<i>P<sub>2</sub>O<sub>5</sub></i> deficient.			<i>N</i> deficient.			<i>K<sub>2</sub>O</i> deficient.			<i>D</i> and <i>H</i> .
	<i>A.</i>	<i>B.</i>	<i>C.</i>	<i>E.</i>	<i>F.</i>	<i>G.</i>	<i>I.</i>	<i>J.</i>	<i>K.</i>	
P.-G.	o	—	—	o	o	o	—	o	o	—
E.A.-G.	o	—	o	o	+	o	o	—	—	—
P.-A.-G.	o	—	o	o	+	+	—	—	o	—
S.-A.-G.	o	o	—	+	o	+	—	—	o	—
E.A.-P.	o	o	+	o	+	o	o	—	—	o
P.-A.-P.	o	—	o	o	+	+	o	—	o	o
S.-A.-P.	o	o	o	+	o	+	o	o	o	+
P.-A.-E.A.	—	—	—	o	o	+	o	o	+	o
S.-A.-E.A.	o	o	—	o	—	o	—	o	+	+
S.-A.-P.-A.	o	+	o	+	o	o	o	o	o	+

+ indicates first variety exceeds second by  $2\sigma$ .

— " second " " first by  $2\sigma$ .

o " varieties do not differ by  $2\sigma$ .

the largest differences in Table IV, i.e. to those included in the values greater than  $2\sigma$ . It can therefore be assumed that such values in Table IV indicate real differences between varieties in their response to manuring.

The most interesting fact brought out is that the varietal supremacy in yielding capacity depends on the particular salt in minimum in the nutrient medium; the variety Goldthorpe shows this effect markedly. This variety is prevaillingly the lowest in amount of ears or straw produced in complete manure, or in nitrogen or phosphate deficiency, and the highest in amount in the potash-deficient series. It will be observed that with increasing deficiency of any one of the manurial constituents the signs remain predominantly unchanged. Where the changes do occur, as in S.-A.-P.-A. in nitrogen deficiency for ears, they show a definite drift, indicating that a varying response of varieties to changes of intensity of manuring occurs as well as to changes in the type of constituent deficient.

The reversal in the potash-deficient series of the order of yield of varieties in the other manurings is the most striking feature brought out by these tables.

(b) *A Comparison of the Varieties with Changing Manuring.*

From Table III there is strong presumptive evidence that the relative yields of the varieties change with the type of manuring, but, as already pointed out, the differences in yield for an isolated pair of varieties are not



statistically significant. It was therefore necessary to make a comparison of the yields of the five varieties simultaneously over a series of manurial types. This was carried out by means of the 'Analysis of Variance'. From the total variance the portions of the variance due to treatment and to varieties were eliminated, and the remaining variance was due to interaction between the factors eliminated—i.e. to a differential response of varieties to differences of manuring. The values in Table II were used for estimating the variances, the natural logarithms of the mean yields being used instead of the mean yields themselves, since, whilst the absolute variance differs markedly in the different treatments, the relative variance is nearly constant. This procedure is the more appropriate since Fisher and MacKenzie (4) found that the interaction of two factors on yield could better be fitted by a product than a summation formula. An example from the calculations is given below for the nitrogen- and phosphate-deficient series taken together for straw.

<i>Group.</i>	<i>Degrees of Freedom.</i>	<i>Sum of Squares of Deviations.</i>	<i>Mean Square.</i>
Treatment	7	50.75234	
Varieties	4	0.03969	
Remainder (differential)	(28)	(0.25589)	0.00914
Total	<u>39</u>	<u>51.04792</u>	

In this case, as in that of the others, over 99 per cent. of the total variance was due to treatment emphasizing the small contribution made to the total variance by varieties, compared with that made by manuring. The mean square value obtained as above is an estimate of the differential response. For a test of its significance an independent estimate of error was made from each manurial type. The natural logarithms of the yields of the replicates were used for calculating the error, to make the value of the error comparable with the value of the differential response. In each manurial type the 29 degrees of freedom available were made up of 4 due to differences between varieties and 25 for an estimate of error. The following calculations were for straw, manuring 'E'.

<i>Group.</i>	<i>Degrees of Freedom.</i>	<i>Sum of Squares of Deviations.</i>	<i>Mean Square per Pot.</i>
Varieties	4	0.46378	
(Error)	(25)	(0.71754)	0.02870
Total	<u>29</u>	<u>1.18132</u>	

The deviations for the 'Total' variance were measured for each pot from the average value of the thirty pots. The deviations of the varietal means from the mean of the varietal means were used for the variance for

'Varieties', six times this value being used for comparison of variances on a single pot basis. The 'Mean Square' value obtained was in turn six times that required for comparison with the differential value, which had been calculated from means of the replicates and not from the replicates themselves (a factor was used instead of six, where necessary). The mean of the values of errors obtained for the manurial types was taken for comparison with the 'Differential' value obtained. The values of 'Z' obtained by this comparison are given for all parts and for the whole plant in Table V, together with the 5 per cent. probability value for the particular degrees of freedom available.

TABLE V.

*Values of 'Z' for Significance of Differential Response.*

<i>Group.</i>	<i>Phosphate-deficient Series.</i>	<i>Nitrogen-deficient Series.</i>	<i>Phosphate- and Nitrogen-deficient Series.</i>
Ears	0.2036	-0.0036	0.1008
Straw	0.0944	0.6266	0.5570
Roots	0.3282	0.2293	0.3535
Total tops	0.0971	0.4124	0.5671
Grand total	0.1855	0.3682	0.4418
5 % prob. value of 'Z'	0.3079 ( $n_1 = 12$ $n_2 = 95$ )	0.3079 ( $n_1 = 12$ $n_2 = 95$ )	0.2130 ( $n_1 = 28$ $n_2 = 190$ )

The nitrogen- and phosphate-deficient series are treated separately and together. It was impossible to include the potash-deficient series owing to the great variability of the I and J types. Inspection of Table II shows marked differences in the yields of the varieties in type K, and had this type alone of the potash series been included, the values of 'Z' obtained would have been considerably higher than those given in Table V. In fact the most obvious contribution to the differential response of the varieties has been excluded by the omission of the potash-deficient series from the comparisons.

# DISCUSSION.

The numerous significant values of 'Z' shown in Table V clearly establish the existence of a differential varietal response to manuring. These significant values were obtained for straw, roots, total tops, and grand total. The ears alone failed to give a significant value. The reason for this failure was the large variability of dry weight of ears found among replicates, particularly in the most deficient types. The sample in these types con-

sisted of very few ears, and the discontinuity resulting from differences in number of ears greatly affected the weights of these small samples. For the purpose of the present investigation the absence of a statistically significant value for ears is unimportant, as the whole plant weights are of more value in studying efficiency in utilizing manures.

It is not intended in the present paper to discuss in detail the nature of the varietal differences. It should be mentioned, however, that differences between the varieties in the potash-deficient series were so great as to be easily visible a month after germination. Though the differences in the potash deficiency, owing to the greater variability, are not statistically significant, it is thus probable that the differential effect is here very marked.

The existence of a differential effect of change of manuring on the yield of varieties, particularly as the effects can be traced through a series of increasing deficiency in one constituent, has a definite agricultural importance, since it indicates the necessity for the combination of varietal trials with manurial trials.

It is not claimed that the pot-culture method can finally establish the agricultural value of these results, but it is confidently expected that when a sufficiently precise technique is applied in field trials the differential varietal response to manuring will be established. Indications of this have already been obtained by Beaven, as mentioned earlier.

The results, further indicate the possibility of breeding directly for efficiency in the use of manure, and for the raising of varieties particularly suited for soil types known to be deficient in some essential constituent.

In a later paper an analysis of the varieties, in terms of their physiological behaviour, will be attempted.

#### SUMMARY.

The experiment described establishes the existence of a differential response of varieties of barley to various types of manuring.

Five varieties were grown with eleven types of manuring, including deficiency of nitrogen, of phosphate, and of potash. Seven replicates of each variety for each manuring were used, requiring the use of 385 pots in all.

The resulting dry-weight data are treated by the 'Analysis of Variance' method (Fisher (3)) and significant values are obtained for the differential response of the varieties to manuring.

The varieties are compared in pairs to indicate the particular varietal differences contributing to this differential response.

The agricultural importance of the results is indicated.

In conclusion, the authors wish to express their thanks to Sir John Russell for facilities for carrying out the experiment at Rothamsted Experimental Station, to Dr. R. A. Fisher for advice in the statistical treatment, and to Professor V. H. Blackman for his interest during the progress of the work.

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# APPENDIX.

I am able to supply confirmation of the general conclusions of the authors from the results of barley experiments at my station at Warminster.

In a field experiment in 1922 there were twenty-seven parallel alternated half-drill strips of each of two races of barley—P.-A. 1 (Plumage-Archer, 1914) and P.-A. 2 (Plumage-Archer, 1924), each originally single plant cultures with the same ancestry (F. 2, 1907). There were four 'manurings' across the half-drill strips with two controls of 'no manure' similarly across the strips—one at each end of the field—324 plots in all.

The yields of dry grain P.-A. 2, stated as a percentage of yields of dry grain of P.-A. 1, were as follows :

Plots 2	N + P + K	%	Maximum S.E. of any Comparison.
" 5	N only	111.0	
" 3	P + K	106.4	
" 4	N + P	98.0	
" 1 and 6	nil	97.8	
		94.3	3.5 %.

The S.E. above quoted is derived from the weights of total raw produce on each of the plots. From many other experiments it may be accepted that this S.E. of raw produce is higher than that of the corresponding dry grain, which was not determined.

The evidence is conclusive that there was 'differential response' in this experiment. The N was in the form of sulphate of ammonia, and in this particular environment P.-A. 2 'responded better' than did P.-A. 1 to sulphate of ammonia, both when it was combined with phosphate and potash and when it was applied alone. In 1923 a similar experiment under other weather conditions on the same area gave different, but less significant, relative responses as between the same two races of barley.

The results of checker-board 'nursery' plots each year from 1910 up to now (generally twenty plots, each of eight races), in which the environment is only variable in respect of such soil heterogeneity as occurs within a few perches, have demonstrated that even these apparently slight differences in environment produce 'differential responses'. I hope before long to publish these results *in extenso*.

It is, in fact, evident that no two races of a cereal 'respond' quite equally to the same environment, and that, as between races, relative yields on unit area, whether of total produce or of seed, are influenced by *all* the environmental conditions and by their interactions. This seems indeed to be a corollary to the doctrine that the survival of races of any species has been mainly dependent on Natural Selection. It is illustrated by innumerable facts, and may be summarized by the aphorism, 'What is food for one race may be, more or less, poison for another race of the same species'.

A large number of observations, preferably on the field scale, will be required before we can disentangle the environmental effects, to which manures are only a contribution, so as to enable us to apply the knowledge so gained to the breeding of races better adapted than existing races to specified conditions.

In the conduct and interpretation of comparative trials of old and new races, whilst fully recognizing the existence of these 'differential responses', we can at present only proceed by the method of sufficient replication and repetition of comparisons, in space and time, under various environments, and by treatment of the results so obtained by statistical methods in order to evaluate their significance.

Furthermore, environmental effects on the quality as distinct from the quantity of the produce must be taken into account.

WARMINSTER, May 1928.

# Physiological Studies in Plant Nutrition.

## I. The Effect of Manurial Deficiency on the Respiration and Assimilation Rate in Barley.

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With sixteen Figures in the Text.

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### INTRODUCTION.

IN view of the great importance of the problem of plant nutrition, and the amount of attention which the relations of plant yield to manurial applications have received in the past, it is surprising how little work has been published dealing with the effects of various manurial constituents on

such fundamental processes as respiration and photosynthesis. Concerning the assimilation rate, the only work of this kind known to the authors is that of Briggs, who found that deficiency of any of the essential elements (Fe, Mg, or K) led to a lowering of the assimilation rate, a result which he interpreted in terms of change in 'reactive chloroplast surface'. It will appear in the sequel that the evidence presented by Briggs is not conclusive, and, indeed, for phosphate and potash starvation does not appear to be in accordance with the experimental data he brings forward. Examination of these data will be undertaken later, when they will be dealt with in the light of the evidence presented in the present paper.

The effect of manurial deficiency on respiration rate does not seem to have received attention from physiologists in the past, though the effect of solutions of various substances on the respiration of mature plant organs has been studied. Thus Lyon (8) determined the effect of placing *Elodea* in solutions of phosphates, and found an increase in respiration rate. Since death occurred within twenty-four hours, it is probable that the toxic action of phosphates due to secondary effects was being studied rather than the part which phosphate ions play in the general metabolic processes.

The aim of the present series of experiments was twofold: (1) to determine the general course of respiratory and assimilatory activities of leaves in succession throughout the life-cycle of plants adequately supplied with the necessary manurial elements in properly balanced proportions; and (2) to determine the modification of the course of respiration and assimilation induced by deficiency in each of the three elements, nitrogen, phosphorus, and potassium.

The investigation here reported finds its place in a wide scheme of work on the physiological effects of manurial constituents, which has been in progress for some years. By the analysis of growth data information has been gained of the net assimilation rate of plants grown in various culture media, and it was felt that the conclusions thus reached should be tested by further work in the laboratory, dealing specifically with the problems of assimilation and respiration under controlled conditions. The general conclusions from growth data which will appear in subsequent papers of this series have been corroborated by the laboratory work, a fact which lends much support to the methods of growth analysis employed and indicates the validity of results obtained by such methods.

#### EXPERIMENTAL PROCEDURE.

The plants used in this investigation were grown in the open air, in sand culture, in glazed pots each holding 30 lb. of sand, as previously described (18). The variety Plumage Archer was used throughout, and

four manurial series were employed, the following amounts of pure salts per pot being added to each set respectively :

	$\text{Na}_2\text{HPO}_4$ .	$\text{NaNO}_3$ .	$\text{K}_2\text{SO}_4$ .
Fully manured	2.52	9.1	1.85
Phosphate deficient	0.504	9.1	1.85
Nitrate deficient	2.52	1.82	1.85
Potash deficient	2.52	9.1	0.20

In addition to all series were added 0.37 gm.  $\text{CaCl}_2$  per pot.  
1.25 gm.  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .

The seeds were sown on 9th May 1927, and germinated on 18th May. On successive days each week the youngest leaf on the main stem which had reached full development was selected, removed from the plants, and taken to the adjacent laboratory in a stoppered glass tube lined with moist filter paper.

The first determinations were made on 8th–11th June, one on each day in the following order : potash deficient, fully manured, phosphate, nitrate deficient. Each week these four determinations were repeated in the order stated, until the last leaf on the main shoot was dying. The last experiment on the nitrate deficient series was carried out on 18th August, those on fully manured and phosphate deficient the following week, while the potash series yielded one further determination. The first experiment in each case utilized the second and third leaves on the main shoot ; the next five weeks provided determinations on the successive leaves from the third to the seventh week ; by the eighth week the eighth leaf was already too mature for use, and the ninth leaf was therefore used, as also for the following experiment in the ninth week, while all subsequent determinations were made on the tenth leaf. The last determinations, therefore, follow the senescent change in activity of the last leaf, rather than the ageing of the plants as a whole.

#### METHOD OF DETERMINING ASSIMILATION AND RESPIRATION RATES.

*The Katharometer.* The apparatus used for determining the amount of  $\text{CO}_2$  given out or taken up by the leaf was the katharometer. This instrument, as adapted to plant physiological measurements, has been described by Waller (15), and a general account is therefore unnecessary here. In several particulars, however, our experience of the instrument differs from that of Waller. Two types of katharometer have been tried, the compensated type (as used by Waller), and the older asymmetrical form, but the former appears to possess no marked advantages over the latter ; it certainly is not compensated, though in construction it may be symmetrical. In order to obtain true compensation, the first requisite is that the electrical parts of the instrument, and particularly the platinum spirals, be properly balanced ; this was not the case in either of the instruments used by us.



Evidence of this was provided by altering the strength of the heating current passing through the instrument, the strength advised by the makers being 120 milliamps. Assume that with this current flowing, conditions are such that a deflection is produced in the galvanometer. If the instrument is properly balanced electrically, and the heating current is gradually reduced to zero, the galvanometric deflection should also simultaneously come to zero; but even in the compensated instrument this did not hold: the current in the galvanometer circuit fell to zero a long time before that in the main circuit, and then reversed, producing a large deflection in the opposite direction; finally it reversed again, coming to zero.

This effect of the asymmetry is unimportant, as the heating current is kept constant; but it has other, and more troublesome, results. Thus, if both arms of the katharometer are connected to the same chamber, containing air, and the pressure inside this chamber is altered, then the deflection changes; this should not be the case in a compensated instrument. Also, very slight changes of temperature affect the deflection markedly. The result of this sensitivity to so many factors was apparent when the katharometer was set up as described by Waller, though with both arms connected to the same air chamber; the zero of the instrument, instead of remaining constant, showed a slight but continual drift—usually in one direction through the day, reversing during the night. Although this was undoubtedly largely the result of temperature change, it was found impossible to correlate it with this with sufficient accuracy to allow of a correction from a temperature record; on some days when the temperature was more uniform than usual the drift would be greater than the average, and vice versa. This drift proved very troublesome, and it was only by enclosing the katharometer in a double-walled asbestos-lined metal box packed with cotton wool, and the leaf chamber in a large volume of water, and working with the whole apparatus in an underground constant temperature room, that it was reduced to reasonable dimensions. Even with these precautions, the zero drift in extreme cases might amount to as much as 4 per cent. of the respiration rate. One obvious method of reducing the relative value of the drift was to enclose the leaves in as small a chamber as possible, but this proved to have strict practical limits, as will be seen shortly.

Another factor which appears to have been more troublesome to us than to Waller is the lag period of the instrument. This will obviously depend on the length of the diffusion path from the leaf chamber to the spiral, and also on the cross-sectional area of that path. The diffusion path used was as short as practicable and apparently shorter than that used by Waller, if it is possible to judge from his published diagram (Fig. 2). The platinum spirals of the katharometer normally have communication with the outside air only by three very fine tubes drilled through the solid brass case; in the compensated instrument used these tubes were very much enlarged, in

order to reduce as far as possible the resistance to diffusion, and consequently the lag of the instrument. In spite of this, the lag period was very long, considerably over an hour being required, after a switch over from light to darkness, before the maximum rate of movement in the opposite direction (that of respiration) was attained. On reversing these conditions, and changing from a state of respiration alone to one of assimilation, the lag was not so great, the maximum speed being attained usually after about forty minutes. This means that the amount of  $\text{CO}_2$  in the chamber at the beginning of an assimilation period must be sufficiently great to allow assimilation to proceed at its maximum rate for at least this length of time. This introduces a limit to the smallness of the leaf chamber compared with the leaf surface used, since if the chamber is very small, the concentration of  $\text{CO}_2$  at the beginning of illumination would be toxic. In practice, a compromise between these two considerations was made, and a chamber of such a size used that the  $\text{CO}_2$  concentration need never rise above 4-5 per cent.

Whether Waller's instrument actually showed a shorter lag period, or whether the rates of respiration and assimilation as measured by him are short of the actual rates, is not quite clear. A study of his galvanometric record of alternate light and dark periods (Fig. 4) shows that he took his readings for respiration rates within half-an-hour from turning off the light, and for assimilation rates after about ten minutes of illumination. If a 'straight-edge' is placed against the three respiration tracings, it is found that the first is irregular (presumably because the instrument had not been left for a sufficient length of time to settle down), while the two last show a continually increasing rate throughout the whole of the dark period; the correct rate of  $\text{CO}_2$  production is presumably not at the point where it has been measured, but would only have been recorded had the respiration been allowed to continue for a longer time than was actually the case. The three assimilation tracings are too nearly vertical to attempt to draw any conclusion from them, but it would seem highly probable that nowhere on the record has the actual rate been reached,  $\text{CO}_2$  becoming the 'limiting' factor before the lag period has ended; in other words, these curves are sigmoid in form, having no straight portion between the two opposite curvatures.

Waller states that, when the atmospheric pressure round one spiral varied while that round the other was constant, a change of '1 per cent. produced a momentary galvanometric deflection which almost immediately subsided, while even with a change of 4 per cent. the deflections were not of much account'. Preliminary observations of the effect of pressure change in one arm of our instrument showed that a change of 1 per cent. produced a deflection (which did *not* subside until the pressure was released) of about 9 mm., i.e. approximately equivalent to that produced by three parts of  $\text{CO}_2$  in 10,000 of air. This appears at first sight considerable, but under

the conditions of working (as Waller has also shown for his apparatus), even assuming as low respiratory and assimilatory ratios as 0.5, the change of pressure caused thereby would only affect the results by 1 per cent. In all probability the error due to such a cause is actually much less than this.

*Calibration.* The instrument was calibrated for  $\text{CO}_2$  by connecting one arm (the one to which the leaf chamber is usually attached) to a large vessel containing air to which has been added a known quantity of  $\text{CO}_2$ . A change of concentration of one part of  $\text{CO}_2$  in 10,000 of air produced a deflection of 3.05 mm. Respiration and assimilation rates calculated according to this calibration are too high, since the effect of the changing concentration of  $\text{O}_2$  has not been taken into account. Assuming that the respiratory and assimilatory coefficients are unity, the results will be 10 per cent. too high, since, according to Daynes, the effect of  $\text{O}_2$  upon thermal conductivity is one-tenth that of  $\text{CO}_2$ , and is in the opposite direction. This correction has been applied to all the results.

*Leaf Chamber.* The leaf chamber finally used consisted of a rectangular block of aluminium with two depressions,  $10 \times 4 \times 1$  cm. deep; over these was placed a piece of glass, the joint being made secure by means of a vaselined india-rubber washer to which the glass was clamped. The depressions thus formed two chambers, the leaves to be experimented upon being placed in one, mounted on a piece of glass which loosely fitted into it; the other was furnished with a similar piece of glass. The chambers were connected one to each arm of the katharometer by means of two tubes let through the walls of the aluminium block, and finally the whole block was sunk in a large water-bath, this latter having a polished glass front.

When this chamber was first used, in running blank experiments, it was found that the galvanometer moved as fast as in a normal respiration experiment. The aluminium was evidently giving off some gas, in all probability hydrogen, to which the katharometer is known to be extremely sensitive. This was largely overcome by painting over the metal surface with shellac, but it was not until a coat of white enamel had been applied that all movement ceased.

The volume of the leaf chamber as actually used in the experiments, together with the air space in the katharometer surrounding the platinum spiral, was determined by removing a known volume of air, and finding the change of pressure caused thereby. The volume thus obtained was 35.7 c.c.

The light used in the assimilation experiments was provided by three Mazda gas-filled bulbs (150 watts, 110 v.), immersed in running water, and placed a few inches from the leaf chamber; the light from these was concentrated by means of a large condenser lens. Although precautions were taken to absorb as much as possible of the heat from these lamps, it was found impossible altogether to prevent them affecting the katharometer.

However, the greatest effect was produced shortly after switching on the light, the drift caused thereby gradually subsiding, until after half an hour it was well under 1 per cent. of a normal assimilation rate. In determining the light intensity at the compensation point (at which assimilation just balances respiration), one lamp only was used, without the condenser lens, and its distance from the leaves was varied until the deflection of the galvanometer remained constant.

In these experiments, portions only of the leaves could be used; strips about  $3\frac{1}{2}$  in. long were cut from the central region, each experiment being performed on at least two leaves from different plants. The cut ends were protected by a film of vaseline, by which means the tendency to lose water in the chamber was largely eliminated. Under these conditions also, no increase in the rate of respiration due to wounding was observed; on the contrary, during the first few hours after cutting the leaves, the rate of respiration usually showed a slight but continual falling off. In one instance some leaves were left in darkness in the chamber for several days, and the respiration rate determined every afternoon. The first day gave the highest rate (13.1), and a falling one, which by the second day had reached 10.5. The following three days showed a gradual rise to 12.3, and the next two a fall to 10.0, after which mould appeared on the leaves. The general form of the respiration curve seems to be that found for leaves under conditions of starvation.

*Experimental Procedure.* The experiments were carried out as follows: the leaves were cut and the apparatus set up at 11 a.m.; it was then left to settle down, five-minute readings of the respiration rate being started at 2.30 p.m. These readings were continued for two and a half hours, after which the light intensity at the compensation point was determined by the method described above. The light was again extinguished, and the  $\text{CO}_2$  of respiration allowed to accumulate until late in the evening. When this had reached a concentration of 4-5 per cent., the assimilation rate at the high light intensity was determined; the rate of movement of the spot of light over the galvanometer scale proved so rapid that it was usually found more accurate to observe the time taken to move equal distances than to take galvanometer readings at equal intervals of time. At the conclusion of the experiment the fresh weight, the leaf area (by means of a planimeter), and the dry weight of the leaves were determined.

The temperature at which the experiments were performed depended on that of the underground room in which the apparatus was housed. Throughout the series the maximum variation was about  $4^\circ\text{C}$ ., and this was more or less progressive through the summer; the respiration and assimilation rates were corrected to an approximate mean value of  $17^\circ\text{C}$ . by using a coefficient of 2.0 for a rise of  $10^\circ\text{C}$ .

Before going on to describe the experimental results it may be well to

give a record of actual values obtained in a typical experiment, in order to give some indication of the accuracy with which estimates of assimilation and respiration rates may be made with the katharometer when sufficient precautions are taken to eliminate sources of error.

Experiment 2. Fully manured series, Thursday, 16th June 1927.

Leaves removed from plants 11.30 a.m., in chamber 12 noon.

<i>Respiration, 5-minute readings.</i>			<i>Assimilation.</i>		
Time.	Reading on Scale.	Rate (Scale Divisions).	Time (Seconds) for a Deflection of 20 mm.		
p.m.					
3.5	167.1	—	53	49	46
3.10	178.8	11.7	52	48	46
3.15	190.0	11.2	53	49	46
3.20	201.1	11.1	52	48	46
3.25	212.7	11.6	51	47	46
3.35	223.8	11.1	50	47	—
3.40	235.0	11.2	50	46	—
3.45	246.0	11.0	51	48	—
3.50	257.1	11.1	50	46	—
3.55	268.3	11.2	49	47	—
4.0	279.5	11.2	49	46	—

The figures show the steady respiration values obtained, while the gradual rise in assimilation rate to a steady maximum value is also apparent. It would seem that accurate measures of assimilation rate could be made in as short a space of time as a minute, and of respiration in five minutes.

#### EXPERIMENTAL RESULTS.

*Various Data.* Before dealing with the respiration and assimilation rates of these leaves, it may be interesting to note the course taken by various other characteristics of the leaves in the four series throughout the season of which the values are given in Table I. The data are given for each of the four types of manuring used for successive leaves on the main shoot throughout the vegetative cycle. These values are presented graphically in Figs. 1, 2, and 4. In Fig. 1 is shown the ratio of dry weight to leaf area. Generally speaking, this is higher in the nitrate and phosphate deficient series than in the fully manured, while it is considerably lower in the case of potash deficiency. The general form of curve is quite similar in all four types, the level being determined by the manuring, and the 'period' of the oscillations about the mean level by the length of the life-history of the plants—this latter itself, of course, being dependent on the manuring. The highest values obtained for the ratio were in the nitrogen deficient series, and the lowest in the potash deficient. The curves in all four cases show a rise during the first week, then a fall to a minimum (the drop occupying one, two, three, and six weeks respectively in the four

TABLE I.

$$\frac{\text{Weight of Water}}{\text{Leaf Area}} = \text{mg. water per dm. Leaf Surface.}$$

$$\frac{\text{Dry Weight}}{\text{Leaf Area}} = \text{weight in mg. per dm.}^2 \text{ Leaf Surface.}$$

Leaf No.	Fully manured.				Potash deficient.				Nitrogen deficient.				Phosphate deficient.			
	Wt. of Water. Leaf Area.	Wt. of Water. Dry Wt.	Dry Wt. Leaf Area.	Wt. of Water. Leaf Area.	Dry Wt. Leaf Area.	Wt. of Water. Leaf Area.	Dry Wt. Leaf Area.	Wt. of Water. Leaf Area.	Dry Wt. Leaf Area.	Wt. of Water. Leaf Area.	Dry Wt. Leaf Area.	Wt. of Water. Leaf Area.	Dry Wt. Leaf Area.	Wt. of Water. Leaf Area.	Dry Wt. Leaf Area.	
2 and 3	205.3	5.31	38.6	239.5	7.57	195.7	31.6	195.7	5.79	33.8	191.6	4.72	40.8			
3	219.2	5.20	42.2	227.8	6.24	212.3	37.2	212.3	5.83	37.0	229.7	5.44	43.3			
4	209.6	6.36	33.4	232.8	6.65	206.2	35.0	206.2	5.88	35.1	252.3	5.91	42.7			
5	234.7	6.84	34.4	256.9	8.73	225.8	29.4	225.8	5.35	42.2	251.9	6.80	37.0			
6	194.7	6.25	31.2	199.9	7.26	163.9	27.5	163.9	3.41	48.1	219.7	5.57	39.5			
7	141.4	3.59	39.4	177.0	6.89	139.3	25.7	139.3	3.02	46.3	148.6	3.19	46.5			
9	146.7	3.50	42.0	154.1	5.76	146.0	26.7	146.0	3.40	42.9	142.1	3.31	42.9			
9	133.2	3.60	37.0	141.4	5.51	124.8	25.6	124.8	2.77	45.0	132.8	3.11	42.7			
10	100.9	2.55	39.7	105.5	3.29	93.2	32.1	93.2	2.27	41.2	92.6	2.22	41.7			
10	99.6	2.58	38.6	114.8	3.38	96.7	34.0	96.7	2.72	35.5	92.9	2.31	40.2			
10	95.4	2.57	37.0	104.7	3.48	89.4	30.1	89.4	2.59	34.5	86.6	2.06	42.0			
10	69.9	2.02	34.6	121.3	3.74	—	32.4	—	—	—	86.8	2.08	41.7			
—	—	—	—	118.8	3.51	—	33.9	—	—	—	—	—	—			

types) followed by a rise to a maximum two weeks later, and a subsequent decline. In the way these curves are plotted, the four maxima lie nearly on a straight line; in other words, the value of this ratio at the maximum is an inverse function of the time taken to reach this stage. With the exception of the phosphate deficient series (and the leaf used in the fourth determination of this series was somewhat abnormal)<sup>1</sup> the minima in these curves also lie on a straight line.

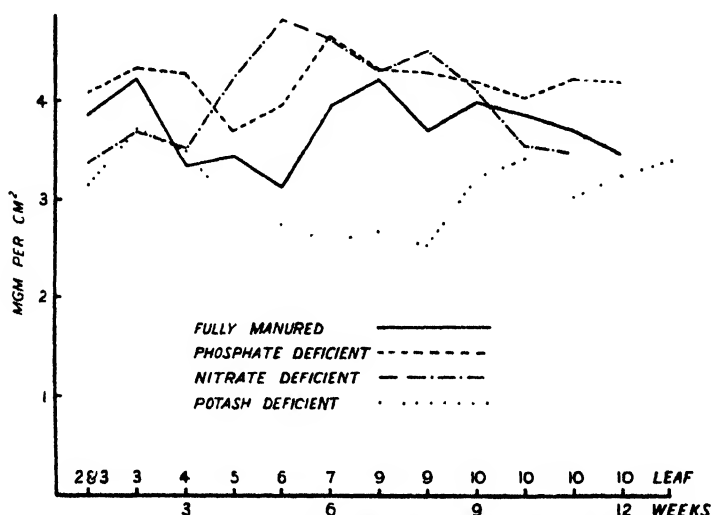


FIG. 1. Graph showing weight in mgm. per square cm. of leaf surface of the leaves of the four series.

The water content showed the usual responses obtained with these types of manuring (Fig. 2). The plants starved of potash had a very much higher, while the phosphate and nitrate deficient had a rather lower, average water content than the fully manured series. Throughout the life-history, in all four types, the general form of curve is fairly constant; there is a rise to a maximum at the fourth week, or fifth leaf (earlier in the nitrate deficient), and a subsequent decline, this latter (with the exception of the potash deficient series) occurring in more or less well-marked steps, chiefly between the sixth and seventh (fifth and sixth in the nitrate deficient) and between the eighth and ninth leaves.

At first sight, there is very little connexion between the water-content curves and the curves for the ratio of dry weight to leaf area (Figs. 1 and 2), but a close inspection shows that the inter-relationship in the water-content curves of any two series is the inverse of the inter-relationship in the dry weight to leaf area curves of the same two series. This is particularly well marked in the case of the nitrate deficient and the fully manured series.

<sup>1</sup> The basal portion of this leaf only was used as the tip was already yellow and dry.

Not only is the nitrate deficient curve generally below that of the fully manured in one case, and above it in the other, but the two pairs cross over twice at identical points, and a close approach in one pair always coincides

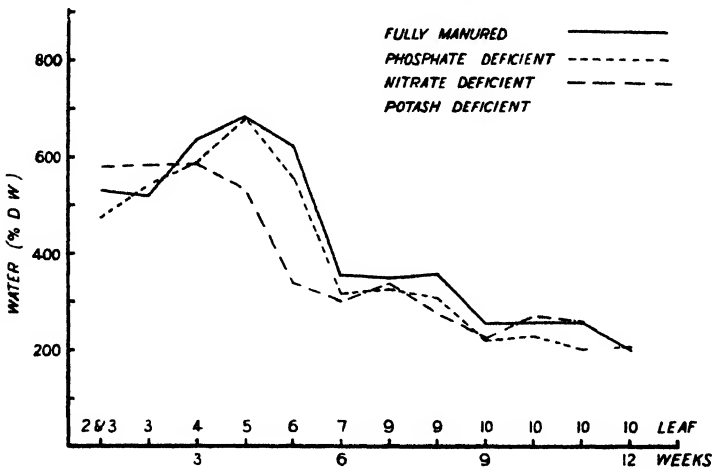


FIG. 2. Graph showing water content as percentage of dry weight in leaves of the four series

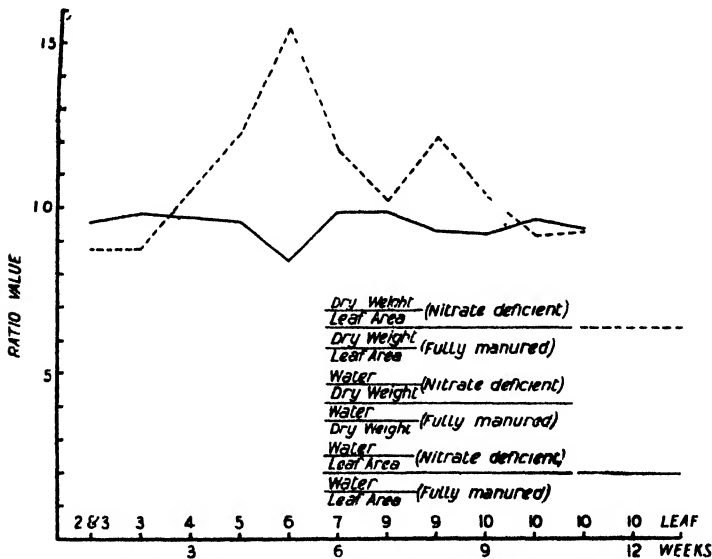


FIG. 3. Graph showing various relationships of water content and dry weight in the fully manured and nitrate deficient series. For description see text.

with a close approach in the other. This relationship in these two manurial types is better shown in Fig. 3, where the ratio of the water content in one series to that in the other is drawn, together with the ratio of dry-weight-per-unit-leaf-area in one to that in the other. The third curve is obtained by multiplying together corresponding points of the other two, and its



approach to horizontality is a measure of the inverse proportion existing between them. Now the product of these other two ratios is merely the ratio between the weight-of-water-per-unit-leaf-area in the nitrogen deficient and that in the fully manured series, which is thus shown to be nearly constant. In the same way, a very high correlation can be shown to exist

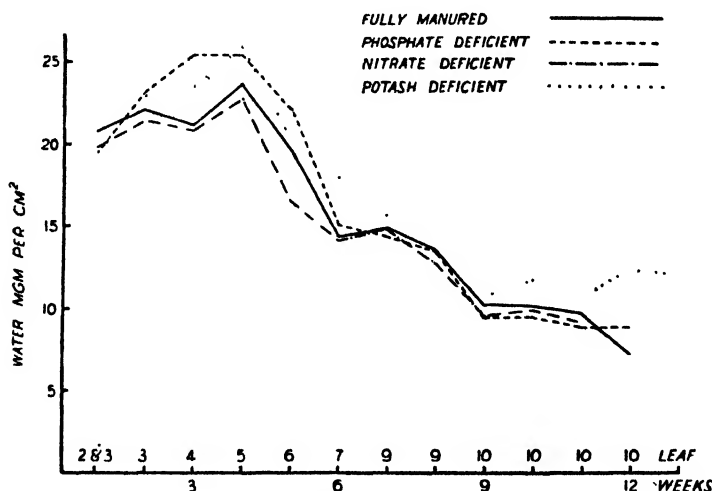


FIG. 4. Graph showing variation of water content (as mg. per cm.<sup>2</sup>) in time in the leaves of the four series.

between the weight-of-water-per-unit-leaf-area in the potash deficient and the fully manured series throughout the season, and a lesser correlation between the phosphate deficient and the fully manured.

Thus, in all four series (with the exception of a few early points in the phosphate deficiency curve) the amounts of water per unit area vary together much more uniformly than do the water contents as measured in the usual way, on a dry weight basis. This is shown in Fig. 4; the general course of these curves is very similar to that of the water-content curves, but the four are much closer together, both in general level and also—at least in the case of the nitrogen deficiency—as to the time of the maximum and the times at which the greatest amounts of water are lost. The potash deficient leaves still have their water content (on an area basis) very slightly higher than the fully manured, and the nitrogen deficient very slightly lower. The only points which are thrown further out are a few of the early ones in the phosphate deficient series; in this case, the water content, instead of being uniformly rather below that of the fully manured, as on a dry weight basis, is at first higher, crossing over and becoming lower at about the eighth leaf.

These considerations appear to indicate that in the case of leaves, area provides a better basis for the calculation of water content than does dry weight.

*Respiration Rate.* The rates of respiration corrected for temperature are presented in Table II, calculated on both the dry weight and leaf area bases. Graphs of these values on a dry weight basis are shown in Fig. 5. When plotted on the basis of leaf area, the general course is very similar, though with a steeper drop in the early portion; the relative positions of the four are also nearly the same, though those of the phosphate and nitrate deficient series are generally raised very slightly compared with that of the fully manured, and that of the potash deficient series is lowered, particularly near the middle part of the curve. Precisely the same differences are brought out as in the curves shown, though to a slightly less degree.

TABLE II.

*Rates of Respiration.*

Respiration on Dry Weight Basis gives mg. CO <sub>2</sub> per hour per grm. Dry Weight								
" " Leaf Area " " " " " sq. dec. Leaf Surface per hour.								
Leaf No.	Fully Manured.		Potash Deficient.		Nitrogen Deficient.		Phosphate Deficient.	
	Dry Wt. Basis.	Leaf Area Basis.	Dry Wt. Basis.	Leaf Area Basis.	Dry Wt. Basis.	Leaf Area Basis.	Dry Wt. Basis.	Leaf Area Basis.
2 and 3	4.49	1.734	5.93	1.881	4.15	1.399	4.95	2.020
3	4.24	1.822	5.33	1.978	3.56	1.319	3.80	1.647
4	2.81	0.937	4.77	1.671	3.26	1.144	4.01	1.713
5	2.77	0.950	4.23	1.243	2.00	0.851	2.64	0.975
6	2.78	0.868	4.20	1.152	1.64	0.789	2.91	1.155
7	2.65	1.042	3.81	0.987	1.62	0.743	1.81	0.840
9	2.38	0.996	4.16	1.109	2.07	0.886	2.28	0.979
9	2.65	0.981	4.56	1.166	1.98	0.891	2.78	1.193
10	2.86	1.133	4.71	1.506	2.31	0.952	3.28	1.364
10	2.26	0.870	4.38	1.492	2.20	0.780	2.72	1.093
10	2.68	0.990	3.80	1.143	2.40	0.829	2.56	1.077
10	2.52	0.873	4.45	1.442	—	—	2.57	1.068
10	—	—	4.47	1.518	—	—	—	—

The general shape of the curve in the fully manured series indicates a rapid fall in the rate of respiration during the first two weeks of the observations, subsequent determinations showing very little further change. In the three deficient series, the initial drop was more protracted, respiratory activity reaching a minimum at the seventh leaf, and subsequently showing some measure of recovery. It should be remembered that all these determinations (with the exception of the last few in each series) were made on the youngest fully-formed leaf, the points giving, therefore, the approximate values of the respiration rates of the successive leaves at corresponding early ages.

The four curves give conclusive evidence that the respiration rate of the young leaves varies with manuring, and that different manurial constituents affect the rate in different ways. On a dry weight basis, phosphate deficiency has no marked effect on the rate, this curve and that of the fully

manured crossing over eight times in twelve determinations. The mean percentage value, compared with the fully manured series, is 103.6 per cent., rising to 116.8 per cent. on a leaf area basis. Nitrate deficiency produces a marked drop in the respiration rate, the only determination in this series

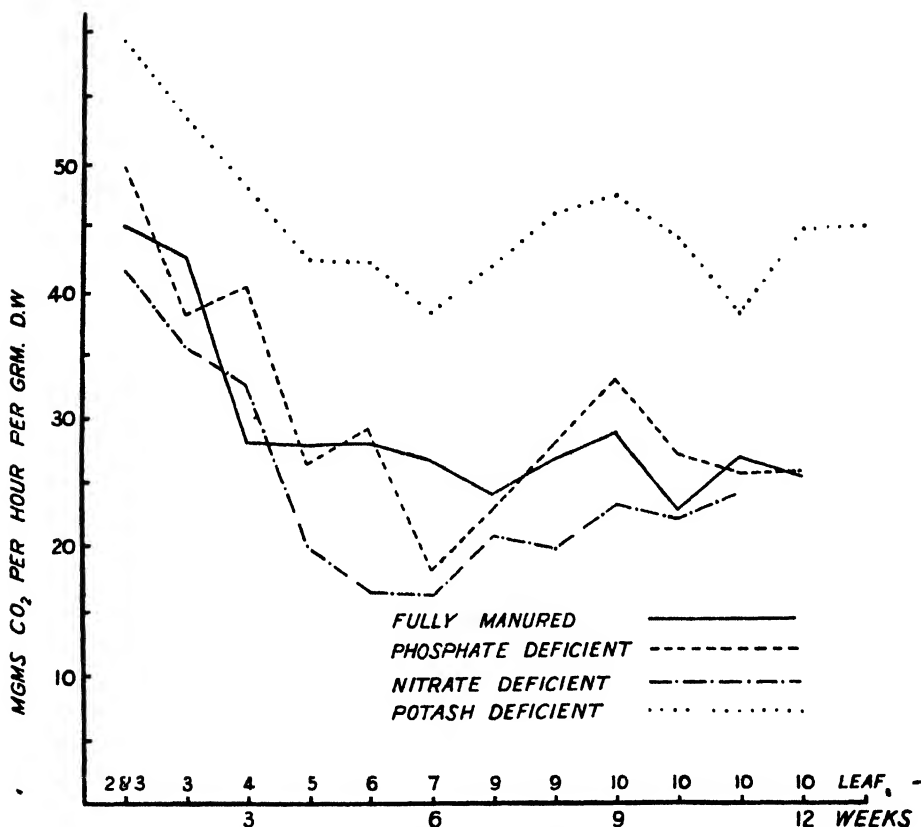


FIG. 5. Graph showing rates of respiration on a dry weight basis of leaves of the four series.

exceeding the corresponding one in the fully manured being the third. The mean percentage value throughout the season compared with the latter was 83 per cent., and 87.7 per cent on a leaf area basis—on this basis, also, the third week was the only one giving results in the reverse order. Deficiency of potash, on the other hand, produces a very marked rise in the rate of respiration, the mean value, on a dry weight basis, being 158.3 per cent. of that in the fully manured series; the rate is well in excess even on a leaf area basis, in spite of the much lower value of the ratio of dry weight to leaf area in this series, i. e. 130.6 per cent., the increase being approximately half that on the dry weight basis. It is interesting to note that, considering the three deficient series, the rates of respiration as determined each week were invariably in the same order—in an ascending series, nitrate, phosphate, and

potash deficient. This is true also for the nitrate deficient, fully manured, and potash deficient, with the single exception at the third determination already noted.

The results which have been presented qualitatively above may also be dealt with quantitatively. There are clearly two factors at work, namely, (1) the effect of age of plant inducing a fall in respiration in all the series, (2) the effect of manurial deficiency modifying the effect of the first factor. In order to disentangle the effects of these two factors, and further to obtain an estimate of error of the determinations, Fisher's Analysis of Variance (4) has been used. To obtain a symmetrical table the first eleven values of each series alone have been employed. In essence the method makes use of the fact that the total variance of the figures about their mean value may be divided into two parts: that due to known causes, in this case age and manuring, and a residue due to unknown causes (the experimental errors). The variance attributable to these causes may then be tested against each other to determine whether the effects obtained differ significantly from the variance to be expected from the unaccountable variation (experimental error). For this purpose, Fisher's *Z* test (4) has been used. The results obtained are given below.

*Analysis of Variance for Respiration Rate.*

(Dry Weight Basis.)

Mean Value of 11 Respiration Rates.

Fully manured	2.961	} Mean = 3.259.
Nitrogen deficient	2.472	
Phosphate deficient	3.067	
Potash deficient	4.535	

	Degrees of Freedom.	Sum of Squares.	Mean Square.	Log Mean Square.	Standard Deviation.
Age	10	21.2733	2.1273	0.75482	—
Treatment	3	26.0952	8.6984	2.1638	—
Remainder	30	2.6584	0.08861	-2.42351	0.2977 (9%)
Total	43	50.0269	—	—	—
<i>Z</i> (Age remainder)			1.5892	1 % point = 0.555	
<i>Z</i> (Manuring remainder)			2.2933	1 % point = 0.753	

The result of the analysis shows very clearly that the variance due to manuring, as well as to age, are significantly different from variance due to experimental error,<sup>1</sup> and the odds are far greater than 100:1 against the result obtained arising by chance. It may therefore safely be concluded that the fall of respiration rate in successive leaves as the plant ages is real, and further, that the respiration rates of leaves from plants differently manured vary with the treatment. The estimate of experimental error from the 30 degrees of freedom of the Remainder makes possible a direct

<sup>1</sup> The 1 % point in the *Z* distribution is the value of *Z* which has for the given number of degrees of freedom a significance of 100:1. Both values of *Z* obtained are of very much higher significance.

test of the differences between the means of the separate manurial treatments. The value of the standard error is seen to be 12.0 per cent. of the mean respiration rate, indicating that two determinations of respiration on similar leaves from two plants identically manured may differ by 24 per cent. without such differences being significant. This result indicates the variability of the material used, and shows that the minor fluctuations on the curves are due only to variability of the material. Caution is thus required in drawing deductions from experiments if only very few determinations are made, since the differences found may be solely due to unavoidable variability of the plant material used. The value of the standard error appropriate for differences between means of groups of eleven will be = 0.0608.

Comparing the manurial treatments in turn with the fully manured the results are as follows:

	Differences of Means.
Fully manured – Nitrogen deficient	+ 0.489 $\pm$ 0.127
Fully manured – Phosphate deficient	– 0.106 $\pm$ 0.127
Fully manured – Potash deficient	– 1.574 $\pm$ 0.127

Nitrogen and potash deficient series thus differ from the fully manured by more than twice the standard error and are thus significantly different in their respiration rates; nitrogen deficient leaves having a lower, and potash starved a higher respiration rate than the fully manured. The value for experimental error obtained from the Analysis of Variance will be a maximal value, since all the secondary interactions of age and manuring, represented by the varying positions of minima on the curves, and themselves a cause of variance about the mean, are included in the experimental error. The *Z* test is the most reliable means of estimating the relative effects of the causes of variance, and the direct test on differences of the means are merely confirmatory.

*Assimilation Rate.* Briggs claims to have shown that with manurial deficiencies, assimilation rate is subnormal both in the photochemical and dark phases. Bearing this possibility in mind, the experiments here described were carried out at two light intensities (Table III), one low enough to ensure that light was limiting. The high light intensity remained constant throughout, while for estimation of assimilation at low light intensity the compensation point was determined, as previously described. The distance of the single lamp from the leaf chamber was recorded at each determination of the compensation point. To convert these measurements of distance into relative light intensities, and also for purposes of comparison with the high light intensity, the actual intensities for the varying distances and the two illuminations were determined in two ways. The first method consisted in enclosing a Hilger linear thermopile in the leaf chamber in the position normally occupied by the leaves, and under conditions exactly as used in

the assimilation experiments. The radiation intensity was measured by means of a galvanometer in series with the thermopile. This method gave comparative results only in so far as the proportion of light radiation and heat radiation transmitted by the water screens remained unchanged. To test this matter further the light intensity was determined directly with a lumeter, placing the standard white surface inside the leaf chamber as before. The curves obtained by the two methods were identical, and hence the energy values were known both in terms of calories per unit area per unit of time, and metre candles.

TABLE III.

Assimilation Rates on Leaf Area Basis : mg. CO<sub>2</sub> per. sq. dec. per hour.

Leaf No.	Fully Manured.		Potash Deficient.		Nitrogen Deficient.		Phosphate Deficient.	
	Low Light.	High Light.	Low Light.	High Light.	Low Light.	High Light.	Low Light.	High Light.
2 and 3	1·368	17·61	1·496	18·39	1·491	18·22	1·530	16·64
3	1·465	20·26	1·241	15·01	1·345	17·75	1·658	20·03
4	1·131	13·87	1·140	13·64	1·448	13·73	1·579	15·54
5	1·219	16·01	0·529	4·49	1·288	11·52	1·540	16·24
6	1·258	12·00	0·544	4·00	1·573	11·93	1·471	14·29
7	1·523	12·05	1·572	12·37	1·443	10·02	1·491	9·63
9	1·515	9·96	1·112	8·60	1·492	12·59	1·453	13·21
9	1·502	12·77	1·500	11·59	1·385	4·80	1·501	14·66
10	1·355	10·56	1·349	9·48	1·193	7·01	1·424	14·96
10	1·377	8·99	1·570	12·09	1·125	9·19	1·477	12·61
10	1·373	12·19	1·334	11·21	0·914	8·51	1·260	10·53
10	0·784	6·08	1·165	10·21	—	—	1·014	8·53
10	—	—	0·614	5·27	—	—	—	—

The figures obtained in the two calibrations are given below :

(a) Linear thermopile.

Distance from lamp	6 in.	8 in.	10 in.	12 in.	14 in.	16 in.	18 in.
Light value (galvanometer deflection)	96·0	66·1	45·9	34·8	26·9	22·1	18·5

(b) Lumeter.

Distance from lamp	6 in.	8 in.	10 in.	12 in.	14 in.	16 in.	18 in.
Light value (foot candles)	83	55	40	29	22	18	15

If the logarithms of the sets of values of the two variables are plotted the curves obtained are very nearly straight lines, and moreover of almost identical slope in the two cases. Thus the tangents of the slopes of the lines are 1·56 and 1·62 respectively. By interpolation in these curves the light intensity at any distance from the lamps was read off, and by conversion of the galvanometer deflection into metre candles (since the intensity of light was too high to read direct on the lumeter), the high light intensity used was found to be 5,000 metre candles approximately, while the light intensity at the average of the various compensation points was found to be 300 metre candles.

In order to make direct comparisons of the assimilation rates at high and low light intensity, the values of assimilation rate at the compensation point were all reduced to the average light intensity at the compensation point. The value of the assimilation rate at this point is known to be equal to the respiration rate, which has been previously determined. A slight uncertainty is introduced by the small variations in temperature in the different experiments, but it is clear that under these conditions light must be the 'limiting factor'. Further, whatever temperature was actually recorded for a particular experiment, the corresponding respiration rate gives at once without correction the value of assimilation rate strictly comparable with those of other experiments, irrespective of the temperatures prevailing at the time they were performed. The assimilation rates at the compensation points were therefore reduced to a common light intensity by a process of simple proportion, the values of respiration uncorrected for temperature being used in each case. The full justification for this procedure will be given later, but at present it will be seen that the only assumption made is that at the compensation point light is the 'limiting factor'; and hence that in this region assimilation rate is proportional to light intensity.

TABLE IV.

		Respiration.	Assimilation.	
			Low Light Intensity.	High Light Intensity.
Fully manured	—		Unaffected by age of plant	Falling with age of plant
Nitrogen deficient	<i>Subnormal</i>		Normal; unaffected by age of plant	Subnormal; falling with age of plant
Phosphate deficient	Normal		Slightly supernormal; <i>falling with age</i>	Slightly supernormal; falling with age
Potash deficient	<i>Supernormal</i>	<i>Subnormal</i>		<i>Subnormal</i>

Table III presents data of assimilation rates at high and low intensities of light calculated on the basis of mg. CO<sub>2</sub> per sq. decimetre per hour. The ratio of leaf area to dry weight given in Table I make possible, if required, a conversion of the data to the basis of dry weight.

The rates of assimilation (real) under conditions of high light intensity for the four types of manuring are given graphically in Figs. 6–8, calculated on the leaf area basis.

(a) *Assimilation Rate at High Light Intensity.* The general form of the curve in the fully manured series is somewhat similar to the respiration rate curve, showing a continual fall in the early part of the season, and becoming almost horizontal after the sixth or seventh determination; the decline in the assimilation rate is therefore more gradual than that in the respiration rate.

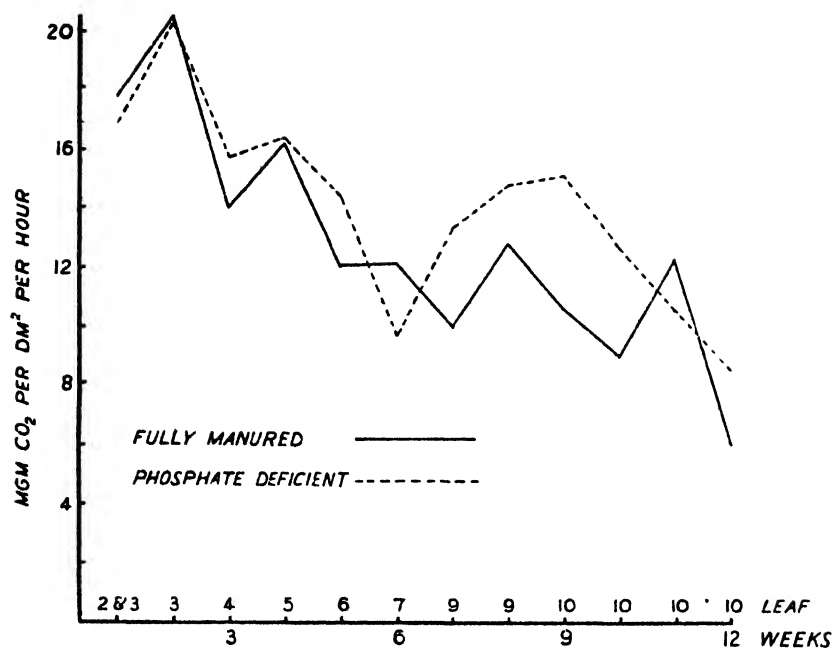


FIG. 6. Graph showing rates of assimilation of leaves of phosphate deficient and fully manured series under high light intensity.

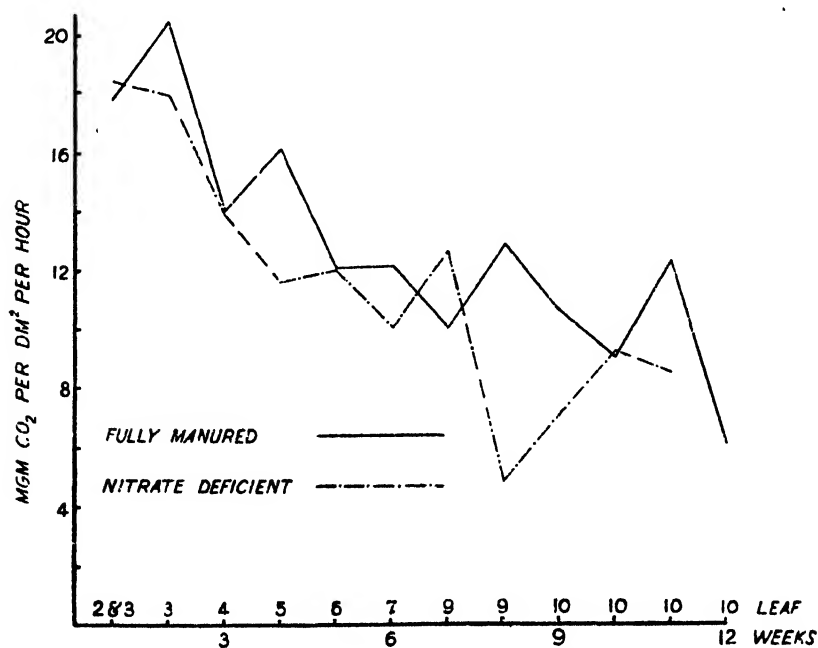


FIG. 7. Graph showing rates of assimilation of leaves of nitrate deficient and fully manured series under high light intensity.



Manurial deficiency apparently affects the rate of assimilation in these young leaves to a much less degree than it affects the respiration rate; but again, the curve of each deficient type shows a definite minimum point, with a subsequent recovery, of which there is no very clear evidence in the fully manured series. Phosphate deficiency certainly does not decrease the assimilation rate, and there is some indication that it causes a slight increase; thus the average percentage rate in this series, compared with the fully manured, on a leaf area basis, is 111.1 per cent.; on a dry weight basis, however, the mean value is 100.4 per cent. (in these and later values the final determinations are ignored, as they are often obviously abnormal, and were made on leaves which were rapidly dying). As can be seen from Fig. 6, there is very little difference between the two rates until after the minimum at the seventh leaf, when the recovery in the phosphate deficient series pulls up its average figure. On a dry weight basis this curve is uniformly slightly below that of the fully manured until the minimum is reached, subsequent points being generally higher—in fact, the inverse relationship to that obtaining in the corresponding curves of the amounts of water per unit leaf area. It is interesting to note that this minimum occurs at precisely the same point as that in the respiration rate curve for this series; both processes subsequently rise to a maximum at the ninth determination, finally falling off together. The general agreements between respiration and assimilation holding throughout the life-histories in the fully manured and phosphate deficient series (and a similar agreement can be traced, though not so well, in the other two deficient series) are interesting in the bearing they may have on the conclusions of such workers as Plester (11), concerning green and yellow varieties, Henrici (6), dealing with alpine and lowland plants, and Boysen-Jensen (1), who find that in plants of the same species, those with a high photosynthetic rate generally have also a high respiration rate, and vice versa. Turning again to the minima in these two rates at the sixth determination of the phosphate series, it may be pointed out that they occur at precisely the same point as the maximum in the ratio of dry weight to leaf area; this, however, is probably unimportant, since the same relationship cannot be traced in the other series. It is more likely to be significant that these minima occur about the time of the cessation of tillering and the beginning of increase in length of the stems.

In the case of the nitrate deficient series (Fig. 7), a slight decrease in the average rate of assimilation is indicated; the mean percentage value of the rate of the fully manured series is 88.2 per cent. on a leaf area basis, and 84 per cent. on a dry weight basis. Here a minimum is not reached until late in the life-history—the eighth determination, while only eleven were possible before the last leaf had withered.

Potash deficiency has a very decided effect on the general shape of the

curve (Fig. 8), but not, apparently, on its mean height. The curve shows a very marked drop in the assimilation rate at the fourth and fifth determinations, with a subsequent recovery to the value of the fully manured series. Apart from this drop, the general course is similar to that of the latter. On a leaf area basis the mean value is 84.5 per cent. of that of the fully

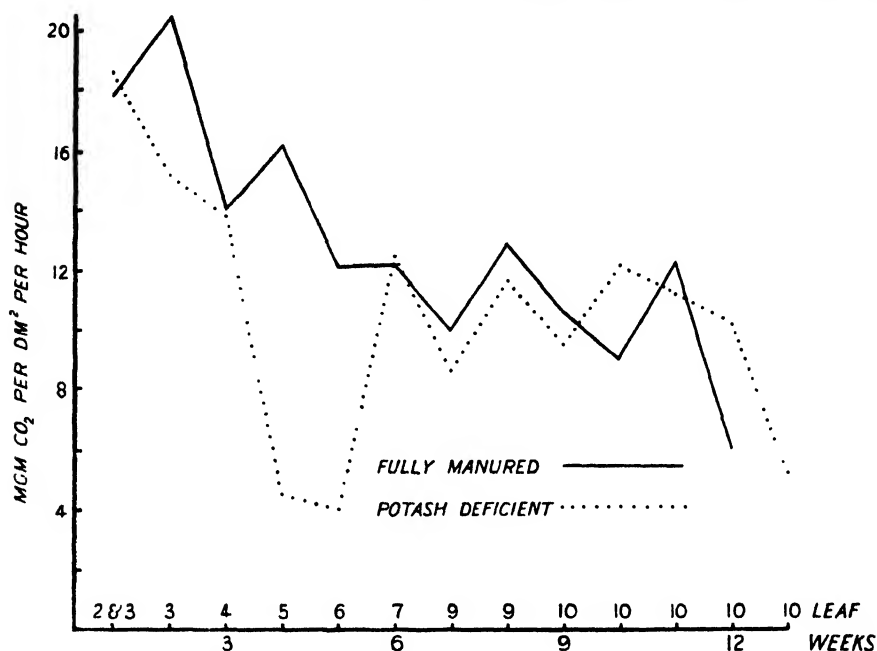
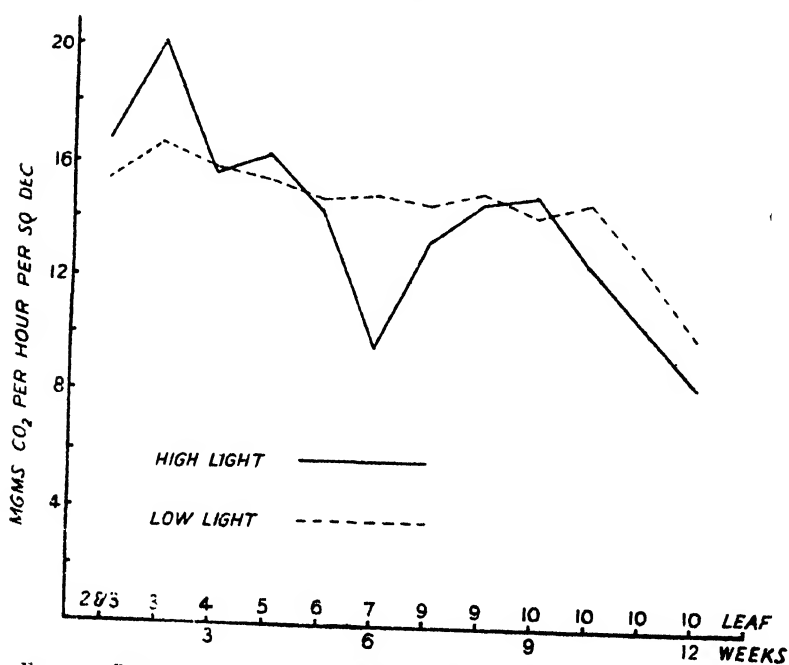
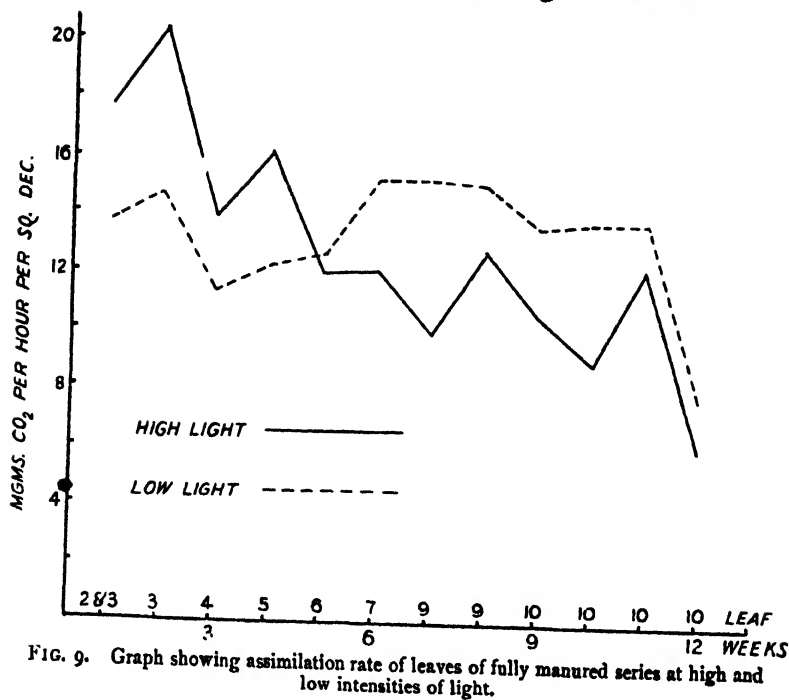


FIG. 8. Graph showing rates of assimilation (mgm. CO<sub>2</sub> per dm.<sup>2</sup> per hour) of leaves of potash-deficient and fully manured series under high light intensity.

manured, or, neglecting the abnormally low fourth and fifth determinations, 96.5 per cent.; thus, apart from this temporary collapse, potash starvation does not depress the assimilation rate to any significant extent. On the other hand, on a dry weight basis, the recovery after this collapse takes the assimilation rate well above that in the fully manured series; even including the two low values the mean is 106.6 per cent. of the latter, and, neglecting them, is as high as 122.6 per cent.

(b) *Assimilation at Low Light Value.* To bring out in the clearest possible manner the relations of the assimilation rates at the two light intensities, they are presented together for each type of manuring in Figs. 9-12. The scale for the low assimilation rates has been increased ten times, a consideration which must be borne in mind in estimating the significance of the fluctuations of the low light intensity curves.

In general for the fully manured series, and the phosphate and nitrogen deficient sets, the contrast between the two curves is very marked. There is no clear fall in assimilation rate at low light intensity in the fully



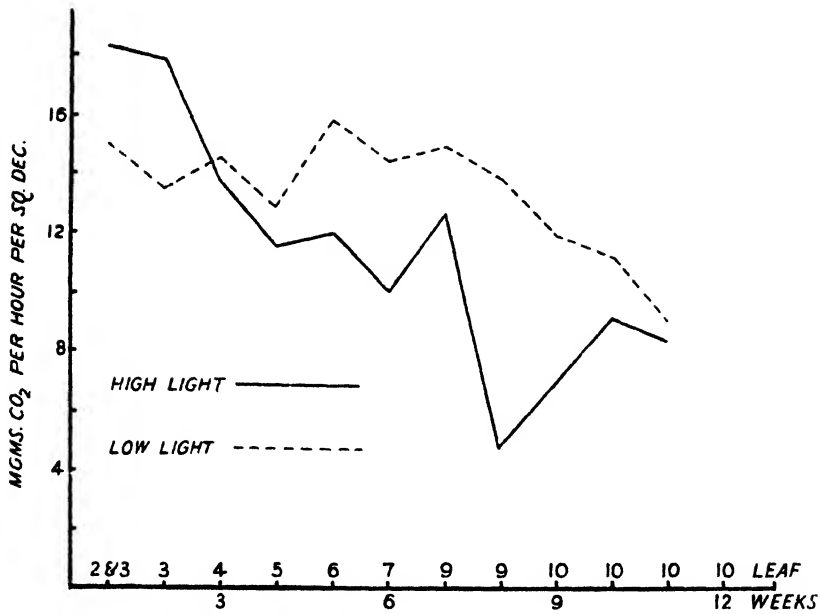


FIG. 11. Graph showing assimilation rate of leaves of nitrate deficient series at high and low intensities of light.

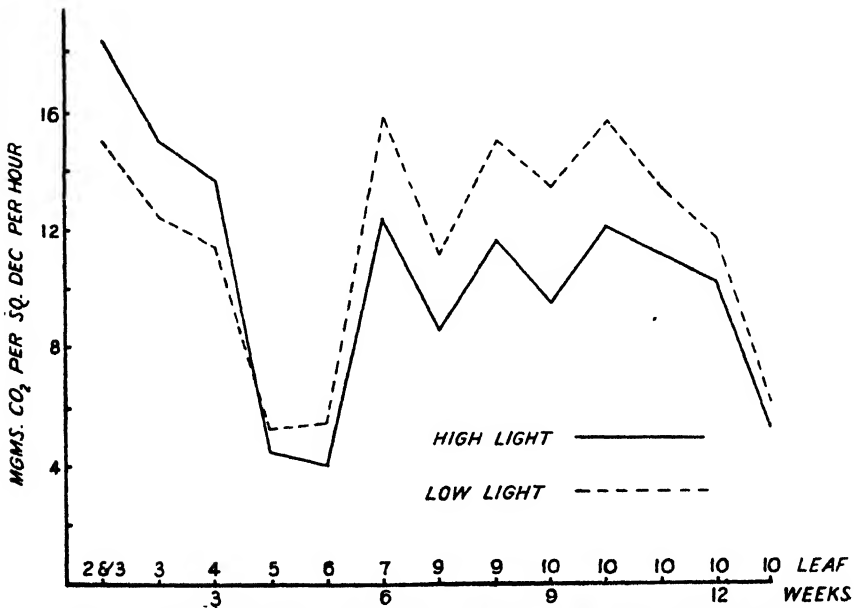


FIG. 12. Graph showing assimilation rate of leaves of potash deficient series at high and low intensities of light.

manured and nitrogen deficient series until the very end, where the values are confined to the last leaf and follow its senescent fall in activity. The phosphate deficient series show a slow but steady decline in assimilation rate at the low light intensity, but to a much less marked extent than at high light intensity.

*Analysis of Variance for Assimilation Rate at High Light Intensity.*

Mean Value of Assimilation Rate for 11 Values.

Fully manured	13.297	} Mean 12.517.
Nitrogen deficient	11.388	
Phosphate deficient	14.395	
Potash deficient	10.988	

	Degrees of Freedom.	Sum of Squares.	Mean Square.	Log Mean Square.	Standard Deviation.
Age	10	338.1926	33.8193	3.52106	—
Manuring	3	85.2007	28.4002	3.34638	—
Remainder	30	215.1559	7.1718	1.97016	2.68 (21.4 %)
Total	43	638.5492	—	—	—

$Z$  (Age: Remainder) = 0.77545

$Z$  (Manuring: Remainder) = 0.68811

1 % point = 0.555

5 % point = 0.5362

1 % point = 0.7531

*Analysis of Variance for Assimilation Rate at Low Light Intensity.*

Mean Value of Assimilation Rate for 11 Values.

Fully manured	1.371	} Mean 1.3535.
Nitrogen deficient	1.336	
Phosphate deficient	1.489	
Potash deficient	1.217	

	Degrees of Freedom.	Sum of Squares.	Mean Square.	Log Mean Square.	Standard Deviation.
Age	10	0.5715	0.05715	-2.86208	—
Manuring	3	0.4152	0.13840	-1.97754	—
Remainder	30	1.4514	0.04838	-3.02867	0.2200 (16.3 %)
Total	43	2.4381	—	—	—

$Z$  (Age: Remainder) = 0.0833

$Z$  (Manuring: Remainder) = 0.5256

5 % point = 0.389

5 % point = 0.536

The series for potash deficiency presents a marked contrast to the others. Here the two curves are very similar in form, and the minor fluctuations agree in their direction in every instance. The marked collapse already referred to in discussing the curves for high assimilation reappear in the curves of low assimilation, and the recovery is shared by both curves to an equal extent. Potash deficiency thus appears to exert a definitely different effect from deficiency in nitrogen and phosphate. In determining the variation in assimilation rates at the two light levels, there are again two main factors at work, namely (1) age effects tending to reduce the assimilation rate with all manurial treatments at high light intensity, but not to such a marked extent at low light intensity, (2) the manurial treat-

ment modifying the age effects in the different series. To disentangle these factors the Analysis of Variance was performed on the two sets of assimilation values, again using the first eleven values alone. The results of the analysis are given in the table opposite.

The results of the analysis throw a very clear light on the interpretation of the curves in Figs. 9–12. The analysis of the data for high light intensity shows that the variance due to age is very significantly greater than that due to experimental error.  $Z$  is considerably greater than its value demands for a significance of 100:1. The variance due to manuring is also very significant and approaches 100:1 probability. In contrast with this the analysis for low light intensity shows that the age effect here is quite insignificant. The manurial effect is, also, insignificant,  $P$  being just less than 20:1. It may therefore be concluded that the fall in assimilation due to age effects appear only when assimilation rate is studied at high light intensity, whereas when light is 'limiting' the assimilation rate the successive leaves have substantially the same assimilation rate. The standard errors of the mean assimilation rates in the two sets are considerably higher than for respiration rate. The reason for this discrepancy is no doubt the fact that in the remainder variance are included the effects of interaction of age and manuring. The different types of curves obtained in the various deficient series indicates that this interaction is considerable, and hence the experimental error is largely overestimated. Testing the differences of the means of the deficient series with the fully manured, using the standard errors discussed, the results are as follows:

High Light Intensity.

Fully manured	– Nitrogen deficient	= +1.909 ± 1.143
"	"	– Phosphate deficient = –1.098 ± 1.143
"	"	– Potash deficient = +2.309 ± 1.143

Low Light Intensity.

Fully manured	– Nitrogen deficient	= +0.035 ± 0.094
"	"	– Phosphate deficient = –0.118 ± 0.094
"	"	– Potash deficient = +0.154 ± 0.094

For high light intensity the potash series alone gives a significant difference, while for the low light intensity none are significant, but potash approaches the significant value. The  $Z$  test, however, leaves little doubt as to the general manurial effect at both light intensities, and it would appear that the series contributing most to the variance is the potash deficient. It is evident that if the differences between the means of the manurial deficient series are taken, phosphate and potash show in both series a significant difference among themselves, thus:

(High Light)	Phosphate deficient – Potash deficient	= +3.407 ± 1.143
(Low Light)	Phosphate deficient – Potash deficient	= +0.272 ± 0.094

To elucidate the meaning of these results it is necessary to digress briefly into a discussion of the interrelations of factors in assimilation.

*Interaction of Factors in Assimilation.* Carbon assimilation is controlled by factors which may be distinguished as external and internal. The action of the external factors has been extensively studied, and their interactions on assimilation successfully formulated by Maskell (9). The nature of the internal factors and their mode of control of assimilation is, however, not so clear. The assimilation process certainly takes place at the surface of the chloroplast, and there seems to be general agreement that the whole process consists of at least five stages:

(a) The passage of  $\text{CO}_2$  from the cell surface to the chloroplast (hydro-diffusion phase).

(b) Combination of  $\text{CO}_2$  with some component of the chloroplast system, and, finally, with the chlorophyll molecule.

(c) Activation of compound molecules by light, chlorophyll possibly acting as photocatalyst, forming some intermediate compound (light reaction).

(d) Chemical reaction (catalysed by an enzyme) among the activated molecules leading to the splitting off of oxygen and formation of the first product of photosynthesis (dark reaction).

(e) Polymerization of the first product to a final product of assimilation (sugar or starch).

The system here summarized is substantially that formulated by Willstätter and Stoll (17), which, on the whole, seems to be best in accordance with experimental evidence. The whole question of the mechanism of photosynthesis is discussed by Stiles (14), who reviews all the literature to date. The course of photosynthesis may thus be represented by a series of linked processes corresponding with the stages enumerated above. If the external factors are all kept at optimal values, i. e. the natural limit of intensity above which secondary disturbing actions appear ( $\text{CO}_2$  narcosis, solarization, temperature time factor), then the rates of each of the linked processes will be determined by the conditions prevailing within the plant (internal factors). The maximal rate of assimilation will thus be determined under optimal external conditions by the internal complex alone. The nature of this complex is largely unknown, although from experimental evidence certain components have been postulated. The process of diffusion into the cell is generally held to be a simple diffusion in imbibed water of the cell-wall and cytoplasm. As will be seen later, this is by no means the only possible or most rapid mode of entry of  $\text{CO}_2$ , and evidence will be presented lending support to a hypothesis of transport of  $\text{CO}_2$  by combination with potassium, in the form of a shifting equilibrium of carbonate and bicarbonate. Potassium may thus be the component of the chloroplast with which combination of  $\text{CO}_2$  first occurs. The velocity of the photo-

chemical process will be determined by four factors: concentration of  $\text{CO}_2$  (or  $\text{HCO}_3$  ion), intensity of light, number of chlorophyll molecules available, and a proportionality factor. The work of Willstätter indicates that the velocity of the reaction is not proportional alone to the mass of chlorophyll present, so that the assimilation number varies within wide limits. Briggs (2), moreover, has claimed that by maintaining the amount of chlorophyll at a low constant level the assimilation rate changed with the age of the leaf, even when light is 'limiting' the assimilation process, in which case there is no question of the efficiency of the dark reaction limiting the rate of the whole process. The evidence for this conclusion of Briggs's is indeed very slender, being based, in fact, on a single experiment with a normal leaf as compared with partially green leaves of *Phaseolus* (Briggs's paper, Table B and Appendix Table VI). Comparison of the figures in Table B of Briggs's paper shows that considerable variation in assimilation rate may occur among individual leaves apart from the stage of greening reached, both at high and low light intensities. If, however, this result is accepted as well established, it is clear that the proportionality factor varies even though the chlorophyll content remains constant. If a curve were constructed showing the relation between assimilation rate and light intensity at low light intensity, where the photochemical reaction controls the rate of the whole process, the resulting curve would be a straight line, and the tangent of slope of this line would be a measure of the proportionality factor. If Briggs's contention is true, the slope of this line would vary with age of otherwise similar leaves, and this slope would be a measure of the efficiency of the photochemical system. Briggs's 'reactive chloroplast surface' is, in fact, nothing but this measure of efficiency, which cannot be estimated except from the slope of the assimilation-light-intensity curve, and his interpretation of it would seem to be purely arbitrary. It is true that a term with the dimensions of area must enter into the function of assimilation rate, but the conclusion that the changing proportionality factor is due to change in this area is unwarranted without further assumptions. The light-intensity-assimilation rate curve for two leaves of different ages (even when light is the limiting factor) may thus be represented formally, as in Fig. 13, A.

The same diagram would equally represent the curves for two different plants varying in reactive chloroplast surface, or in efficiency of photochemical activity. The seasonal variation in assimilation rate for Cherry laurel, even when light limited, led Blackman and Matthei to postulate a 'protoplasmic factor' in assimilation. It is not clear why Briggs (2, p. 257) limits the application of this term to the chemical phase. Applied to both phases it would seem to be a measure of the proportionality factor similar to 'reactive chloroplast surface', both being terms describing the experimentally determined differences in photosynthetic efficiency.



The chemical or dark reaction will depend in rate on four factors: concentration of molecules concerned (activated molecules from the photochemical reaction), temperature, concentration of the final product (first product of photosynthesis), and the velocity constant of reaction. If it is assumed that the final product is removed rapidly when compared with the

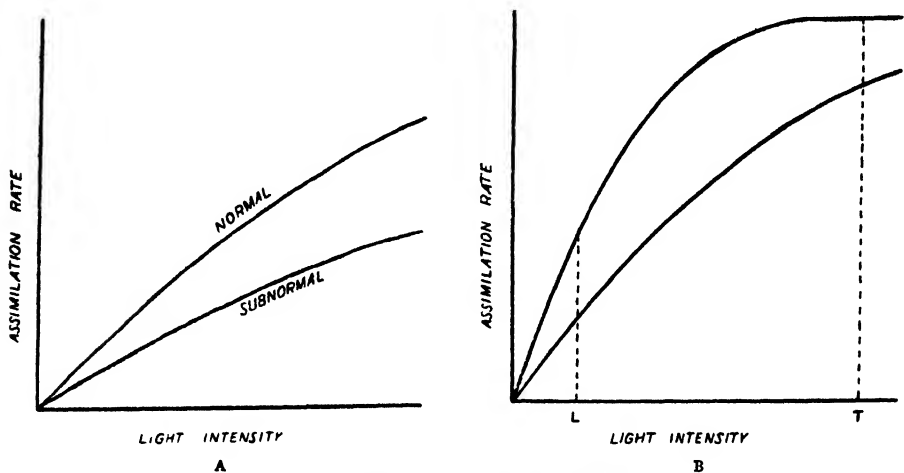


FIG. 13, A and B. For description see text.

reaction under consideration, the rate of the process will be determined solely by the concentration of activated molecules from the photochemical phase and the velocity constant. As the temperature is raised a velocity in the dark phase will finally be reached, at which the number of activated molecules interacting equals the number formed by the photochemical reaction. At this temperature assimilation is light limited. It is clear that with the above system, as postulated by Briggs, the temperature limited values of assimilation rate must be completely determined by the concentration of activated molecules, and hence by the velocity of the photochemical reaction, and therefore with two leaves differing in rate of photochemical reaction the light limited as well as temperature limited values of assimilation rate must be directly proportional to these rates. Since the proportionality factor of photochemical reaction has been called 'reactive chloroplast surface', it follows from Briggs's assumption that 'any change in the amount of reactive surface per unit of leaf will necessitate a change in the photosynthetic activity both when light and when temperature is limiting'. It seems possible, however, that the 'protoplasmic factor' is directly concerned with the dark phase (in fact, Briggs limits the concept to activity in this phase), and, if, as Willstätter assumes, the dark phase is activated by an enzyme, then the velocity of reaction in the chemical phase is not only proportional to the concentration of activated molecules and temperature but to the concentration of enzyme present, and the pro-

portionality factor will now be no longer determined by the 'réactive surface' in the photochemical phase. That such an activation occurs is clear from Willstätter and Stoll's experiments with green and yellow leaves (17, Figs. 7 and 8, p. 149). The results can be formally represented, as in Fig. 13, B.

The ratio of assimilation rates at the light limited values ( $L$ ) to that at temperature limited ( $T$ ) is widely different, and, moreover, varies all over the range of light intensities. That Willstätter's values for 25° C. in Figs. 7-8 are temperature limited at intensity  $I$  is clear by comparison with his Figs. 9-10 where assimilation rate is still rising at 30° C. Figs. 9-10 show clearly that the temperature coefficients differ in the two cases (although light is the limiting factor, as can be seen from the values in Fig. 9 compared with those of Fig. 7 with double light intensity), and, as discussed above, Briggs's assumptions would lead to an identical temperature coefficient in each case. It would seem, therefore, that the 'protoplasmic factor' is of a more complex nature than is envisaged by Briggs's 'reactive surface', and that 'doubling the "reactive surface" should double the activity when temperature is limiting' is, indeed, inherent in the assumptions but not substantiated by experimental fact.

From the foregoing discussion it is evident that it is not necessary to anticipate that the ratio of light-limited and temperature-limited rates should be constant, or that in leaves from plants deficiently manured the subnormality in the light limited rate should be the same as that in the temperature limited. On the contrary, manurial deficiency may act specifically on the efficiencies of the photochemical and dark reactions leading to diverse types of 'subnormality'. Before going on to discuss these specific deficiency effects it is well to be clear as to the meaning of the term 'subnormality'. As has been shown above from the Analysis of Variance, there is in all the series investigated a fall of assimilation rate with age of leaf. The assimilation rate of the leaf depends, therefore, on the age of the plant at the time at which the leaf develops, as well as on the age of the individual leaf. Later formed leaves therefore display 'subnormality' with respect to the earlier leaves, and, as has been shown in the case of the fully manured plants, this subnormality is confined to the rate of assimilation at high light intensity. It is presumably the dark reaction which is affected by the age of the plant as a whole, and the difference may be attributed to differences in enzymatic equipment. A similar fall in respiration has also been established, and equally indicates a fall in activity of enzymes, since the carbohydrate content of the whole plant is presumably rising, and hence fall in respiration cannot be put down to fall in concentration of respirable material. This effect of age confined to the high light values of assimilation is seen equally clearly in Briggs's experiments with *Phaseolus* (his Table VII) in which, for the fully manured plants, the light limited values for leaves of different ages is constant (2.57, 2.56, 2.79 mg.),

while the temperature limited values fall rapidly (5.71, 3.95, 3.35).<sup>1</sup> In dealing with the problem of subnormality this effect must be borne in mind, and leaves of similar morphological age alone must be compared. The method of Analysis of Variance allows this effect to be disentangled from the effect due to manuring, and, as has already been shown, the manurial effect is significant at high light, and almost so at low light. intensity; it would certainly be so if the variance due to the interactions of age and treatment were deducted from that given for the Remainder.

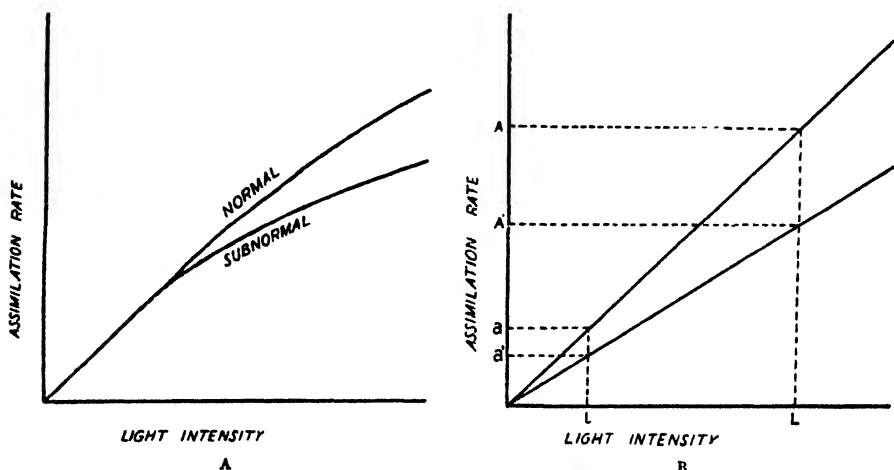


FIG. 14, A and B. For description see text.

The two types of subnormality may be represented formally by the Figs. 13, A, and 14, A, the former showing the typical age effect, as found in the fully manured plants, and Fig. 14, A, the type found in potash and to a less extent in phosphate starved plants.

The experimental results obtained in this investigation will now be examined with a view to establishing the type of subnormality found in the various deficient series.

*Specific Manurial Effects on Subnormality.* Since only two points on the curves at the two light intensities are available, it is only possible to elucidate the types of assimilation curves for successive leaves by indirect means. If the curves are of type 14, A, however, the assimilation rate at high light intensity should be independent of the low light rate, i.e. if the mean of the rates at high light intensity are taken, then a positive deviation of the value of a particular leaf from the mean should not necessarily be associated with a value above the mean at low light intensity for the same leaf. Correlation coefficients were therefore calculated between maximum assimilation and minimum assimilation rates for pairs of values of all the leaves investigated.

<sup>1</sup> The value 3.35 has been substituted for 3.95 given in the table which is obviously incorrect as can be seen from Table V where 3.35 is given, and confirmed by the per cent. values in Table VII.

The results are given below :

*Correlation Coefficient of Maximum Assimilation and Minimum Assimilation Rates.*

Fully manured	$r = -0.111$
Nitrogen deficient	$r = +0.403$
Phosphate deficient	$r = +0.742$
Potash deficient	$r = +0.776$

The values for phosphate and potash deficiencies are highly significant ( $P > 100:1$ ), while for fully manured and nitrogen deficiency the values obtained are quite insignificant.

An objection may be raised at this point on the ground that the assimilation rate at low light intensity has been calculated from the respiration rates and the known light intensities at the compensation point, assuming direct proportionality between light intensity and assimilation rate at this low light intensity. To test whether such proportionality exists the correlations between respiration rates (uncorrected for temperature) and the light values at the compensation point have been calculated with the following results :

*Correlation Between Respiration Rate and Light Value at the Compensation Point.*

Fully manured	$r = +0.928$
Nitrogen deficient	$r = +0.875$
Phosphate deficient	$r = +0.955$
Potash deficient	$r = +0.005$

The uniformly highly significant ( $P > 100:1$ ) and high values of the correlation coefficient in all cases except the potash series, indicates clearly the proportionality existing between light value and assimilation rate, and the near approach to linearity of the assimilation rate curves at low light intensity. These correlation coefficients are not significantly different when the effect of time is eliminated. In all cases but that of the potash series the reduction of assimilation rate to a uniform light value (the mean of the values at the compensation points) by simple proportion would therefore introduce no significant errors into the estimates of low assimilation rates utilized for correlations with high assimilation rates given above.

The approach to rectilinearity of the assimilation curve at low light intensity may be seen from the graphical representation in the phosphate deficient series given in Fig. 15, together with the regression line calculated from the data. In the case of the potash deficient series the low value of the correlation coefficient indicates that even round about the compensation point the assimilation rate is not directly proportional to light intensity. In this case the justification of the reduction of assimilation rates to a uniform light intensity by simple proportion rests on another consideration. It has already been shown in Fig. 12 that the fluctuations in assimilation

rate at high and low light intensities are closely similar, in fact the ratio  $\frac{\text{assimilation at high light}}{\text{assimilation at low light}}$  approaches a constant value. The low correlation between respiration rate and light intensity at the compensation point, already referred to above, is, however, negligibly small, indicating that the curves of assimilation rate and light intensity for successive leaves in no part overlap, but have different slopes even near the origin; in fact, are of the form schematically represented in Fig. 14 B.

If  $a$ ,  $A$  represent the assimilation rates at two light intensities  $l$ ,  $L$  respectively for leaves of one age and  $a'$ ,  $A'$  represent the assimilation rates for leaves of a different age at the same light intensity, then  $a/A = a'/A' = l/L$ . In these experiments  $L$  is the high light intensity which is kept constant,  $l$  the low light intensity which is the light value at the compensation point, while  $a$  is the respiration rate uncorrected for temperature, and  $A$  the observed assimilation rate at high light intensity. The theoretical considerations just discussed may therefore be tested by calculating the correlation coefficient of  $\frac{\text{Respiration}}{\text{Max. assimilation}}$  and the light value at the compensation point. This has been done for each set of experimental values, with the following result:

Correlation	$\frac{\text{Respiration uncorrected for temp.}}{\text{Max. assimilation rate}}$	and light value at compensation point.
	Fully manured	$r = +0.151$
	Nitrogen deficient	$r = +0.210$
	Phosphate deficient	$r = +0.728$
	Potash deficient	$r = +0.900$

Both the phosphate and potash deficient series give highly significant correlation coefficients. It may therefore be assumed that for any single leaf in the case of the potash deficient series no serious errors will be introduced in reducing all the low assimilation rates to a mean light intensity by simple proportion. It is therefore clear that the method used for reducing the minimum assimilation rates to the mean light value at the compensation point is amply justified. The correlations for the phosphate deficient series call for further comment. It has been shown above that the correlation coefficient of respiration rate (uncorrected for temperature) and the light value at the compensation point is highly significant ( $r = +0.955$ ), and further that the correlation coefficient  $\frac{\text{Respiration uncorrected for temp.}}{\text{Max. assimilation rate}}$  and light value at the cp. point is also highly significant ( $r = +0.728$ ), thereby contrasting with the potash deficient series in which the first correlation is insignificant ( $r = +0.005$ ), while the second is very highly significant ( $r = +0.900$ ). Thus for phosphate deficiency the respiration rate is almost proportional to the light value at the compensation point (Fig. 15), and,

further, the ratio of respiration to maximum assimilation is also approaching proportionality with the light value at compensation point. In other words,  $R/L$  approaches constancy, also  $R/L \times A$  approaches constancy ( $R$  = respiration rate uncorrected for temp.,  $L$  = light value at c.p.). Now  $R/L$

$$= \frac{\text{assimilation rate reduced to mean light value at the c.p. (a)}}{\text{mean light value at compensation point}}$$

therefore for the second relation to hold  $a/A$  must approach a constant value,

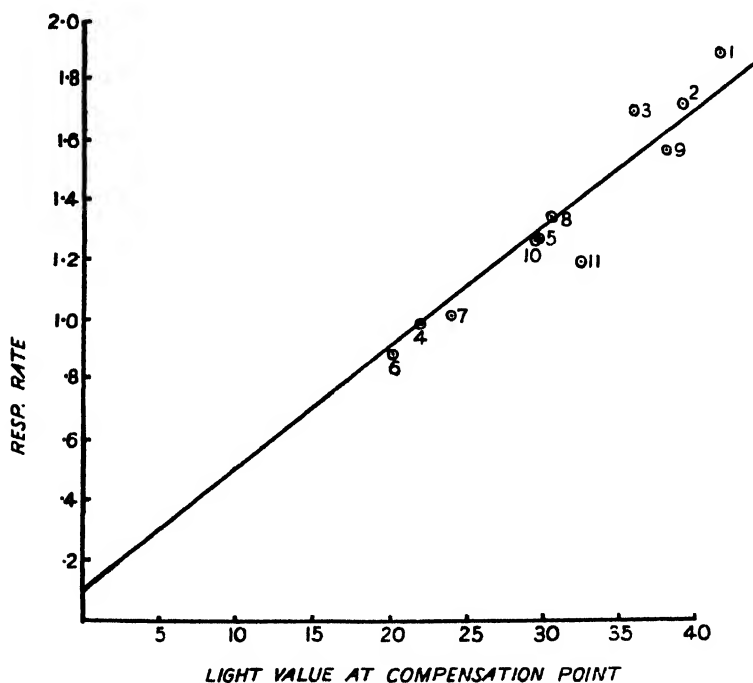


FIG. 15. Graph showing respiration rates and light values at the corresponding compensation points.

in other words, max. and min. assimilation must be highly correlated. Reference to these correlation values given above confirms this, and, further, the value of  $a$  ( $R/L \times \text{constant}$ ) is remarkably uniform in this series. The correlation value of  $a$  and  $A$  indicates that any changes in  $a$  are associated with changes of  $A$  in the same direction, and a reference to Fig. 10 shows that the curve for the two assimilation rates fall together. If this drift is the main cause of the high association, it might be expected that the fall in low assimilation rate curve with time should be significant. In fact, the correlation values of low assimilation rate and time for the phosphate series is highly significant ( $r = -0.796$ ), whereas for the other series it is quite insignificant (F. M. + 0.276, N. def. - 0.502, K. def. + 0.238). It is thus evident that the age effect in the case of phosphate deficiency affects

the assimilation rate even at low light intensity. Since this ageing effect is in part due to falling  $P_2O_5$  concentration in the leaves, it is seen that as the concentration falls the assimilation falls even at low light intensity. Further, the highly significant correlation of respiration rate and maximum assimilation ( $r = +0.681$ ) indicates that phosphate deficiency affects respiration as well as assimilation at both high and low light intensity. The evidence suggests that phosphate is concerned in maintaining the level of assimilation efficiency (active chloroplast surface) as well as respiratory activity.

The effects of manurial deficiency established above are summarized in Table IV (p. 136).

From the table it is clear that the effect of each manurial constituent is specific, and that only in the case of potash deficiency is the assimilation rate subnormal both at high and low light intensities.

*The Effect of Potash Deficiency on Respiration and Assimilation Rate.*

Potash deficiency seems to have an effect differing from that of deficiency in nitrogen and phosphate, in that the assimilation is subnormal both at high and low light intensity. It is already known from previous work (unpublished data) that in the cases of plants grown either with full or deficient manuring of nitrogen and phosphorus the percentage of these elements in the successive leaves decreases. This question and its bearing on growth processes will be discussed in a later paper. At present it may suffice to state that this effect is invariably seen whatever the level of manuring applied, and is a direct consequence of the fact that nitrogen and phosphate control the tillering rate, and that tillering proceeds until internal starvation supervenes. In conditions of nitrogen and phosphate deficiency tillering is slower and is arrested at an earlier stage, so that the internal concentrations of nitrogen and phosphate are automatically adjusted, and internal starvation is no more acute than it eventually becomes in the highly manured plants. Potash deficiency, however, is found to have a much less marked effect on tillering, which proceeds at nearly the same rate as in fully manured plants, with the consequence that internal starvation for potash becomes very acute. This is reflected in the characteristic behaviour of the plant, whose leaves become increasingly yellow, a stage corresponding in time with the rapid collapse in assimilation rate; tillering, however, still proceeds, even when the plants apparently are about to die. Rapid death of tillers now occurs, and death also on the lower leaves on the tillers. After this the new leaves which appear on surviving tillers are darker green, and from this time onwards death of leaves keeps pace with new development, so that at most two living leaves are found on each tiller. This recovery of the plants coincides with the recovery in assimilation rate which becomes nearly normal. It would seem probable that the death of leaves and tillers sets free the potash which is then translocated to the developing leaf. Chemical analyses of the

single leaves used in this investigation were therefore undertaken<sup>1</sup> and the results are presented in Table V.

TABLE V.

Leaf No.	Potash Deficient.			Fully Manured.		
	% K <sub>2</sub> O in Dry Wt.	K <sub>2</sub> O (mg.) per sq. dm.	$\frac{K_2O}{\text{Water Content.}}$	% K <sub>2</sub> O in Dry Wt.	K <sub>2</sub> O (mg.) per sq. dm.	$\frac{K_2O}{\text{Water Content.}}$
2 and 3	1.03	3.26	1.36	2.43	9.39	4.66
3	0.69	2.52	1.11	2.03	8.52	3.89
4	0.65	2.19	0.94	2.35	7.70	3.76
5	(0.67)	(1.98)	(0.75)	0.97	3.32	1.42
6	0.41	1.12	0.56	2.06	6.44	3.30
7	0.37	0.95	0.54	1.08	4.24	3.00
9	0.29	0.76	0.49	1.90	7.95	5.43
9	0.40	1.03	0.73	1.17	4.34	3.26
10	0.30	0.96	0.90	2.21	8.76	8.68
10	0.40	1.36	1.18	2.40	9.25	9.29
10	0.54	1.63	1.56	2.00	7.41	7.78
10	0.27	0.87	0.72	1.67	5.76	8.25
10	0.29	0.99	0.84	—	—	—

The figures in the above table show the large differences found in the potash content of leaves from deficiently manured, as compared with the fully manured, plants. Each series shows also a rapid decline in the potash content in the early stages of growth while tillering is proceeding, reaching a minimum, and subsequent recovery, as the potash, liberated by the dying parts of the plants, is translocated up to the later formed leaves. Except for the fifth leaf in the fully manured series, which seems quite erratic, the percentage of potash in the dry weight for this series, remains higher than for any value in the deficiency series. The weight of potash divided by the weight of water in the leaf is also given in the table. This figure gives a measure of the concentration of potash in the leaf, assuming that all the potash is present in solution. The values of this ratio, together with respiration rate and weight per unit area of leaf, are given in graphical form in Fig. 16. The correlation between the three curves is very striking. There would seem here to be a contradiction with the findings given earlier in the paper, where it was shown that respiration in general was higher in the potash deficiency series, whereas here it is seen that as the potash content of the leaf changes, the respiration varies in the same direction. The relation is, however, entirely spurious as the following correlation coefficients show.

Correlation potash concentration ( $x$ )  
and respiration rate ( $y$ )

$$r_{xy} = + 0.509$$

Correlation potash concentration ( $x$ ) and  
dry weight per unit area of leaf ( $z$ )

$$r_{xz} = + 0.521$$

<sup>1</sup> The authors wish to record their thanks to Dr. Janet W. Brown for performing these difficult analyses.



Correlation respiration ( $y$ ) and dry  
weight per unit area ( $x$ )

$$r_{yx} = +0.859$$

Partial correlation of potash concen-  
tration ( $x$ ) and respiration ( $y$ )  
eliminating ( $z$ )

$$r_{xy \cdot z} = +0.142$$

It is apparent that if the effect of weight per unit area is eliminated, i. e. taking the hypothetical case of leaves of equal weight per unit area but of varying potash content, the potash concentration is without significant effect on the respiration rate. The relationship between weight of unit area of leaf and respiration rate is itself interesting. Since weight per unit area depends on the mass of dead tissue and the mass of cell contents, and the former can have no conceivable effect on respiration, it would seem that the major part of the changing weight per unit area is due to the mass of cell content. Presumably then in this case the weight per unit area is a measure of the mass of protoplasm present, and the effect of decrease in nitrogen in lowering respiration rate is presumably due to the same effect of mass of protoplasm working in the reverse direction. The falling respiration of the whole plant (7), which may be correlated with falling nitrogen content (12), is presumably due also to the falling mass of protoplasm per unit dry weight of the plant. A similar high correlation between respiration and weight of unit area of leaf is seen in the fully manured series ( $r_{yz} = +0.545$ ), whereas the correlation between respiration and potash content is here quite insignificant ( $r_{xy} = -0.161$ ). It would seem, therefore, that potash concentration has no direct effect on respiration rate, but indirectly affects it by determining the level at which other constituents of the cell are maintained.

The correlation coefficient of potash concentration and assimilation rate at high and low light intensities, respectively, eliminating the effect of time, are  $r = +0.591$  for high intensity,  $r = +0.374$  for low light intensity. The first of these values is significant. The original rapid fall in assimilation rate corresponds with the initial rapid fall, and the recovery is accompanied by a rise in potash concentration. The value for the seventh leaf is, however, greater than for the fully manured, while the potash concentration continues to fall for two further weeks before the rise begins. The high correlation between maximum and minimum assimilation rates indicates, however, that some factor is at work determining the efficiency of the photosynthetic process. It is difficult to correlate this with anything but the potash content of leaves. The initial fall in assimilation rate is equally marked at low as at high light intensity, thereby contrasting with the course of assimilation in any other series, in which the assimilation at low light remains almost constant during the initial rapid fall at high light intensity. The possibility presents itself that potash concentration plays a part in the diffusion of  $\text{CO}_2$  into the cell up to the chloroplast surface. Some evidence for this is found in the work of Willstätter, who found that

dry leaf material of certain plants will readily take up  $\text{CO}_2$ , an effect shown by Spoehr and McGee (13) to be associated with the ash content of the leaf, and due to the formation of bicarbonates. It is not suggested that

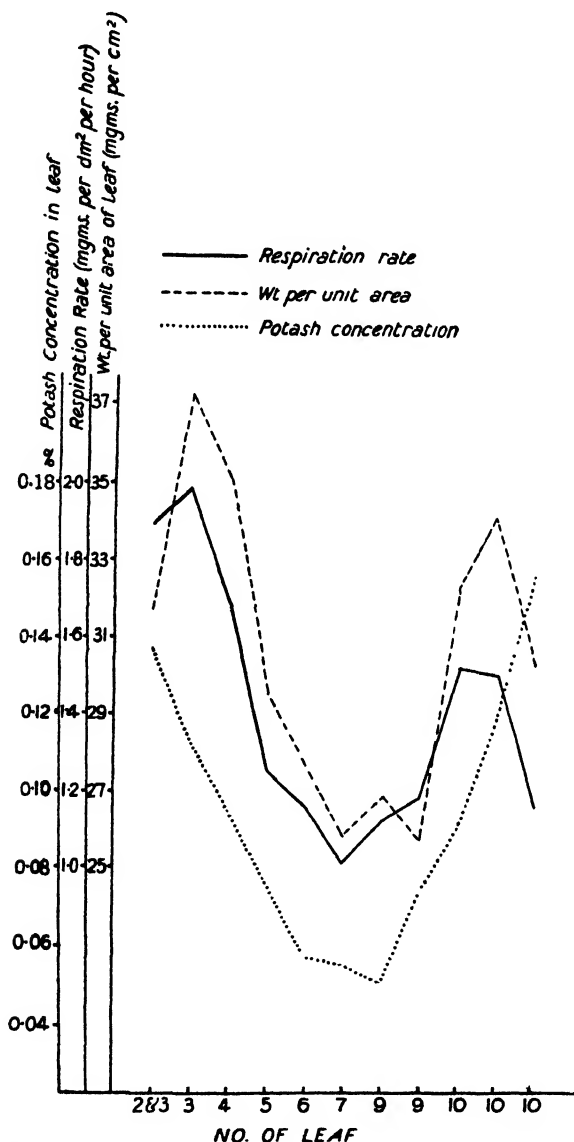


FIG. 16. Graph showing for successive leaves of the potash concentration, the respiration rate, and the weight per cent. area of leaf.

potassium alone could subserve this role, in fact either sodium or calcium are capable of forming bicarbonate. Potassium, however, has two peculiarities which would particularly lend themselves, in that (1) the percentage of potassium in the total ash is very high (as much as 50 per cent. in the

leaves analysed, whereas sodium only occurs in small percentage in most leaves), and (2) potassium is apparently very mobile within the cell, and may be washed out of living cells, indicating that the cytoplasm is extremely permeable to potassium ions. Further work is now in hand which aims at elucidating more clearly the relation of potassium manuring to assimilatory capacity of barley plants, and hence it is not intended without further substantiation to labour this suggestion. In these experiments external  $\text{CO}_2$  was kept so high (4–5 per cent.) that gaseous diffusion into the stomata and up to the external cell-wall may be considered to have introduced a very small resistance into the whole path. The concentration at the intercellular wall surfaces may therefore be considered constant, while the concentration at the chloroplast surface is determined by the light intensity leading to disappearance of  $\text{CO}_2$  at this point. The rate of passage of  $\text{CO}_2$  will thus be determined by the difference between the concentration levels at the surface of the cell and the chloroplast, divided by a resistance factor, itself determined in magnitude by the concentration of the potassium ions present. If light is the 'limiting factor' the falling concentration from the surface of the cell to the chloroplast surface will be proportional to the light intensity, and for leaves differing in potassium concentration the rate of passage of  $\text{CO}_2$  will tend to be proportional to the potassium concentration if this is low. With increasing light intensity, therefore, the assimilation rate would be subnormal in the 'low potash' leaf all over the range of light intensity, and, moreover, the subnormality at high and low light intensity would be proportional. A further deduction would follow, namely, that for low potash leaves for a given light intensity temperature limitation would occur at a lower temperature than for a high potash leaf, or, in other words, the temperature coefficient of assimilation in the higher temperature regions would be *higher* for high potash than for low potash leaf. From work now in hand these deductions will be capable of experimental test.

#### GENERAL DISCUSSION.

In reviewing the results put forward in this paper two considerations must be borne in mind: firstly, deficiency in nitrogen and phosphate was less acute than in potash; secondly, it is not certain whether at high light intensity the assimilation was temperature-limited. Dealing with the first consideration it may be stated that this difference in manuring was deliberate, since nitrogen and, to a less extent, phosphate have been found from previous work to exercise a very marked controlling influence on growth, whereas equivalent doses of potash affect growth to a less marked extent. It seemed desirable to avoid carrying the level of manuring so low that very abnormal plants would be obtained, since it would then be difficult to compare the direct measurements of assimilation rate with those of net

assimilation rate obtained from growth data in previous years. The fact remains that all the typical specific symptoms of starvation were very marked in the plants used; thus the nitrogen starved plants had many fewer tillers than the fully manured, the leaves were a much lighter green and differed characteristically, as was shown in section three of this paper. The phosphate starved also had fewer tillers, and the leaves after the third showed the characteristic reddening associated with phosphate starvation, as well as more pointed and rolled type of leaf. In the fourth determination of this series the green base of the leaf was used, although the upper part of the leaf was orange in colour and almost devoid of chlorophyll. In spite of this the assimilation rate of the green part was apparently unaffected, and not until a fortnight later, when leaves wholly green were used, did the photosynthesis reach a minimum. The potash starved plants also presented a very characteristic appearance, having very yellow thin broad leaves, marked inhibition of stem growth, and completely sterile ears. It is remarkable, therefore, that the deficiency effects obtained differ so markedly from Briggs's findings with *Phaseolus*, for in the latter case the leaves are expressly stated to have been normal in appearance, except for the iron starved, which were chlorotic, and the nitrogen starved plants, for which, however, no assimilation data appear.

Real assimilation rates were obtained by Briggs by adding to the apparent assimilation a 'value well established for normal leaves of *Phaseolus*, namely, 1.5 cc. per gramme dry weight per hour at 15° C.'; without experimental evidence it would seem unlikely that the cotyledons and first pinnate leaves of the plants used by Briggs actually had the same respiration rate. In any case Briggs ruled out entirely the possibility that manurial deficiency had an effect on respiration rate, an effect which the present series of experiments has shown to be considerable, i. e. as much as 60 per cent. in potash starved on leaf area basis, and up to 95 per cent. on dry weight basis above the fully manured. Regarding Briggs's results collectively they appear to show subnormality due to manuring, and an Analysis of Variance performed on his data confirms the impression. In order to obtain a symmetrical table the two phosphate results were omitted, leaving twelve available results. For the low light intensity the variance due to manuring ( $Z = 0.5930$ ) was found to be quite insignificant (5 per cent. point = 0.7798), while for the high light intensity a very significant effect of manuring is apparent ( $P > 100 : 1$ ). The evidence from Briggs's data thus indicates a real subnormality at high light intensity, but no such significant effect at low light intensity. Further, the age effect is very highly significant at the high light, and quite insignificant at the low light, intensity ( $Z = 0.413$ , 5 per cent. point = 0.819). Briggs's results therefore confirm those found in these experiments, in so far as the age effect is much more marked at high light intensity where also subnormality preponderates.

When the individual results are compared it is clear that the lack of iron has greatly affected the assimilation rates at both light intensities, and there is a steady decrease in rate with successive leaves. As far as the two series comparable with the present experiments are concerned the evidence is not so clear. In the case of phosphate deficiency the first simple leaves showed no decrease in assimilation rate, and the first pinnate leaves gave rates of 84.8 per cent. and 82.5 per cent. of the values obtained from a similar single experiment with fully manured plants. As a third experiment is not reported, the evidence would depend on a single comparison, and amount to a reduction of approximately 16 per cent. In the case of potash deficiency the first simple leaf showed a slight reduction (87 per cent. and 96 per cent.) below the fully manured, but the first pinnate leaf (when shortage was presumably more acute) gives values identical with the fully manured. The onus of proof thus rests on the third experiment. In Briggs's Table VII (Summary of Results) the values at high and low light intensity are 67 per cent. and 83 per cent. of those given for fully manured plants. In Table V, however, a third value for yet lower light intensity is given for leaves of both series, and here the potash starved value is 80 per cent. of the fully manured. These three values (80 per cent., 67 per cent., 83 per cent.) should, if Briggs's theory is correct, be identical, and the occurrence of an intermediate value differing so markedly from the two others, indicates that possible errors in technique are involved. The difference in this series of values is of the same order as between the manurial deficiency series and the fully manured control. Further, another source of variation, namely, that due to individual differences in plants similarly treated, has not been considered, and as the present author's experiments show these may be very considerable. Analysis of Variance on Briggs's data has shown that for the high light values the standard deviation after eliminating known sources of variation is 10 per cent., and for the low, 22 per cent. It is thus clear that, from such few determinations as Briggs made, it would be rash to conclude that as far as phosphate and potash starvation are concerned there is any real subnormality at all due to manurial deficiency, apart from the possible relative effects on light-limited and temperature-limited assimilation rates.

In the experiments reported in this paper it is doubtful if the assimilation rates at the high light intensity were temperature limited. As previously stated the light intensity used was 5,000 lux, while Briggs claims to have worked at 36,000 lux. It should be made clear, however, that the light intensity (5,000 lux) here used was experimentally determined; had the intensity been calculated merely from the candle power of the lamps, it would have been rated at a much higher value. Thus the three half-watt lamps used totalled 450 watts, and were at a distance of 8 in. from the leaf chamber; this, assuming as low an efficiency as one candle per watt,

would give a *calculated* value of 10,000 lux. It is therefore evident that calculated values of light intensity are suspect. Willstätter states that he calculated the light intensity, while Briggs gives no clue as to how the light intensities in his experiment were estimated. Even though the high light values were not temperature limited, it would be difficult to reconcile the results obtained in the present experiments with Briggs's contention of the 'active chloroplast surface'. It would appear that even the efficiency of light-limited assimilation varies with light intensity. The simplest explanation would be that with rising light intensity the assimilation rate is more and more controlled by the rate of the dark reaction, a result which is easily explicable on the assumption of a reversible reaction in the photochemical phase. Under these circumstances the concentration of the intermediate compounds (which is the substrate of the dark reaction) would depend on the relative velocities of both reactions. With rising light intensity the concentration of the intermediate compound would tend to rise, which would result in increase in rate of the dark reaction, until the rate of formation of the intermediate compound just equalled the rate of removal by the dark reaction. In such a system both reactions would control the rate of assimilation at all light intensities, and the 'partial limitation' would depend on the relative velocities of two reactions. That such a reversible reaction is concerned is indicated by Warburg's experiments with intermittent light (16).

Finally, it may be pointed out that the results here recorded corroborate previous work done by one of the present authors (5), in which net assimilation rates in the early stages of the life cycle up to maximum leaf area are shown to be independent of the level of nitrogen manuring maintained. In this case assimilation is limited by  $\text{CO}_2$  supply, since the plants were grown in the open air, and, apparently, under these circumstances the assimilation rate remains constant and is unaffected by age, just as was found for nitrogen deficient and fully manured plants in the present experiments when light was limiting. In both these cases the assimilation is controlled almost completely by the level of the external factors, the internal factors (efficiencies of the light and dark reaction) being sufficiently high to maintain assimilation rate at a value determined by the level of the external factors. Unpublished work with phosphate manuring has shown that the net assimilation rate under outdoor conditions falls continuously if phosphate level is low, so that, as in the present experiment, phosphate shortage would appear to lead to 'subnormality' even when assimilation rate is limited by external factors (light or  $\text{CO}_2$ ).

In conclusion the authors wish to thank Sir E. J. Russell for granting facilities for the work to be performed at Rothamsted, and Professor V. H. Blackman for his ready interest and helpful criticism.

## SUMMARY.

1. The use of the katharometer for the measurement of respiration and assimilation rates of leaves is discussed ; methods are indicated of overcoming some of the difficulties in its use.

2. The effect of deficiency in nitrogen, phosphorus, and potash on water content and weight per unit area of successive leaves of barley, as compared with those of fully manured plants, are studied ; they lead to the conclusion that leaf area is a better basis than is dry weight for the expression of water content of leaves.

3. Respiration rates for successive leaves of nitrogen, phosphorus, and potash deficient plants as compared with fully manured plants are given. In all the deficient series, the rate of respiration falls to a minimum with a subsequent rise ; while in the case of the fully manured plants the rate falls rapidly at first, becoming constant later. Nitrogen starved plants are shown to have a consistently lower respiration rate than fully manured, potash a consistently higher rate, and phosphate to be unaffected. The differences found are as follows :

	Differences of Means.
Fully manured – Nitrogen deficient	+ 0.489 $\pm$ 0.127
Fully manured – Phosphate deficient	– 0.106 $\pm$ 0.127
Fully manured – Potash deficient	– 1.574 $\pm$ 0.127

Analysis of Variance shows that the effect of age of plant and manurial deficiency are both very significant ( $P > 100 : 1$ ).

4. Assimilation rates at known high and low light intensities are given for successive leaves of fully manured plants, and also for each of the deficient series.

It is shown that changes in assimilation rate are due to the action of two factors, namely, age of plant and manurial deficiency. By Analysis of Variance the following facts were established :

(i) At low light intensity the effect of age is quite insignificant, but the manurial effect is almost significant, due predominantly to the value of the potash deficient series.

(ii) At high light intensity the effect of age is very highly significant ( $P > 100 : 1$ ), and the manurial effect is also very significant.

The bearing of these results on the nature of the 'internal factor' in photosynthesis is discussed. It is shown that two types of subnormality occur, namely, that due to age and that due to manurial deficiency. Subnormality of later formed leaves, as compared with earlier, is found in all the series.

5. Subnormality due to manurial deficiency is found to be specific in effect for the various constituents. The results obtained may be summarized thus :

	Respiration.	Assimilation.	
		Low Light Intensity.	High Light Intensity.
Fully manured	Normal	Unaffected by age of plant	Falling with age of plant.
Nitrogen deficient	<i>Subnormal</i>	Normal; unaffected by age of plant	Subnormal; falling with age of plant
Phosphate deficient	Normal	Slightly supernormal. <i>Falling with age</i>	Slightly supernormal. Falling with age.
Potash deficient	<i>Supernormal</i>	<i>Subnormal</i>	<i>Subnormal</i>

6. The subnormality due to potash deficiency is further discussed, and its theoretical bearing indicated.

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# Physiological Studies in Plant Nutrition.

## II.<sup>1</sup> The Effect of Manurial Deficiency upon the Mechanical Strength of Barley Straw.<sup>2</sup>

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With five Figures in the Text.

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### INTRODUCTION.

IT was at one time believed that the strength of cereal straw, and therefore its resistance to 'lodging' by wind and rain, depended upon the silica content of the straw. It is, however, now realized that the supply of salts available to the plant and the atmospheric conditions of light and humidity under which it is grown are the factors controlling this property. For example, an excessive supply of nitrogen results in a sappy type of growth, the tillers being easily 'lodged'. Experiments at Rothamsted have shown that this effect is reduced by the addition of potassium salts, and that large doses of nitrogen can safely be given provided the nitrogen-potassium ratio is maintained at a normal level.

Several investigators have attempted to determine if the marked effects of varying manurial conditions are correlated with changes in the anatomical structure. They agree that large doses of nitrogenous manures

<sup>1</sup> Part I of this series appeared in this Journal, Vol. XL.

<sup>2</sup> Thesis submitted for M.Sc. degree of the University of London.

result in weakness, potassic and phosphatic fertilizers having the reverse effect; but no clear alterations in the anatomy were found to be caused by different manurings. The earlier investigators, Dassonville (1), and Kissel (2), obtained inconclusive results. Purvis (3) examined the effect of potash deficiency upon the anatomy of *Dactylis glomerata* from the Rothamsted grassland experiments. Although no measurements are given, she decided that no differences in the relative development of the various tissues were caused by varying the supply of potash. From measurements of the thickness of the cell-wall and the diameter of the lumen, it was shown that early in the season the ratio of diameter of lumen to thickness of cell-wall was increased by increase of potassium, but that this effect disappeared later. The conclusion was reached that the strengthening effect of potassium is not due to its effect upon the gross anatomy of the plant.

Advantage was taken of plants remaining from a large-scale experiment upon the effect of manurial deficiency on the physiology of barley plants to obtain further evidence on this subject.

#### EXPERIMENTAL METHODS.

The basis of the method employed was the measurement of the force required to crush portions of the straw of barley plants grown under varying manurial conditions, and the correlation of the strength thus found with the thickness of the tissues present. The barley employed was selected Plumage Archer, grown in the open in sand culture in glazed pots each holding 30 lb. of sand. The plants germinated on May 18, 1927, and were examined during the latter part of August and the first week of September. Four types of manuring were employed, one being 'complete' and the remainder being deficient in nitrogen, phosphorus, and potassium, respectively. The plants were grown three in a pot, each pot receiving the following weight (gram.) of salts.

	$\text{Na}_2\text{HPO}_4$	$\text{NaNO}_3$	$\text{K}_2\text{SO}_4$
Fully manured . . .	2.52	9.10	1.85
Nitrate deficient . . .	2.52	1.82	1.85
Phosphate deficient . . .	0.504	9.10	1.85
Potash deficient . . .	2.52	9.10	0.20

Each pot also received 0.37 gram. of  $\text{CaCl}_2$  and 1.25 gram. of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .

The plants were examined at approximately the same stage of their morphological history—just after the ears had 'shot'. Each plant was uprooted and placed in water in the laboratory. The large tillers in which the ear had 'shot' to a uniform extent were cut from the plant as required and the internodes numbered from above downwards, the peduncle being neglected. As a rule only five internodes of each tiller were large enough for experiment, the lower ones being very small and bearing numerous

adventitious roots upon the nodes. From each internode several portions of stem from 1.0 to 3.0 cm. in length were cut with a sharp scalpel, the majority being between 1.5 and 2.5 cm.; 1 cm. on each side of the node was neglected. At the same time thin sections were cut with a razor from varying positions in the internode and preserved in 50 per cent. alcohol.

Each portion of stem was placed at a constant distance from the fulcrum of two hinged plates, from the upper of which a brass rod projected. On this rod ran a small pulley-wheel by means of which a weight of any desired magnitude could be drawn slowly along the rod by a thread attached to the pulley. By this means the portion of stem was subjected continuously to a gradually increasing pressure. Crushing usually took place sharply. The distance of the pulley from the fulcrum when crushing occurred was then measured.

Then if:

$S$  = the force in grammes required to crush 1 cm. length of tiller,

$D$  = distance of the weight from the fulcrum in cm. when crushing occurred,

$d$  = distance of the portion of the stem from the fulcrum in cm.,

$W$  = weight in grammes moved along the beam, i. e. the weight of the pulley plus that of the suspended weight,

$l$  = length of the portion of stem in cm., and if  $w$  = weight in grammes of the beam acting upon the stem,

$$S = \frac{WD}{dl} + \frac{w}{l}.$$

$w$  was found by measuring the force required to just raise the rod by attaching a spring balance at the distance of  $d$  cm. from the fulcrum. By testing several portions of each internode, an average value of  $S$  for each internode of each tiller examined was found.

It was found desirable, when dealing with weak stems, to use a lighter apparatus; two hinged similar plates but without a rod upon the upper were therefore used, the weight being gently slid by hand over the upper plate until crushing occurred.

#### EXPERIMENTAL RESULTS.

The average force required to crush unit length of each internode of barley plants grown under varying manurial conditions is shown in Table I, the number of internodes upon which the values are based being shown in brackets. Those values which differ significantly from the corresponding values in fully manured plants are printed in black type. By the use of Fisher's (4) table of ' $t$ ' it is possible, knowing the number in each sample and the variation within the samples, to estimate the probability that the observed differences between the means should occur by chance between

means of samples drawn from plants, which have been similarly treated. Where the probability against a difference of that magnitude occurring by chance is greater than one in twenty, the difference is accepted as 'significant'.

TABLE I.

Force in grm. wt. required to crush 1 cm. length of straw.

Internode numbered from peduncle downward.	Fully Manured.	Nitrogen Deficient.	Phosphate Deficient.	Potassium Deficient.
1	229 (7)	241 (10)	151 (3)	231 (11)
2	347 (8)	445 (10)	306 (8)	508 (11)
3	465 (5)	786 (9)	572 (8)	764 (10)
4	771 (7)	1,604 (10)	1,000 (8)	813 (6)
5	1,407 (6)	2,543 (6)	2,097 (8)	698 (4)

The strength of the stem in the fully-manured plants increased rapidly from above downwards. The effect of nitrogen and phosphorus deficiency is to render this still more marked, as is shown by the large increase in the strength of the lower internodes, while the strength of the upper internodes approximates to the normal, i. e. to that of the fully-manured plants. Potassium-starved plants, however, show an interesting departure from this. The uppermost internode is normal but the middle internodes show a strength above normal. The fourth and fifth internodes are, however, weaker than the normal, the fifth internode being only half as strong as that of the fully-manured plants; the significance of this difference is greater than 100:1. In the cases of the fifth internode of the nitrogen and phosphorus-deficient series the chances are more than the 100:1 and 20:1 respectively against the differences from the fully-manured plants being due to chance. The difference between the third internode of the fully-manured and potassium-deficient series is high, but is prevented from being significant by an aberrant observation of 184 grm. among the data on which the mean value of 764 is based. The data of Table I are shown graphically in Fig. 1. The belief of the practical agriculturist in the weakening effect of nitrogen and the strengthening effect of potassium is borne out by these figures.

It will be noted that the effect of mineral deficiency appears least in the upper internodes, becoming progressively more marked down the stem. The potassium-starved plants are weaker in the lowest internodes than in those just above, in sharp contrast to the other three types.

#### ANATOMICAL RELATIONSHIPS.

The anatomy of the barley stem follows the usual grass type. Beneath the epidermis occurs a zone of sclerenchyma, having a more or less wavy

outline internally. Embedded in this are small islands of unthickened chlorenchymatous cells. Between this and the schizogenous central cavity is a band of parenchyma, of which a variable amount abutting upon the sclerenchymatous zone possesses thickened and lignified cell-walls. Scattered throughout the tissues are the vascular bundles descending from

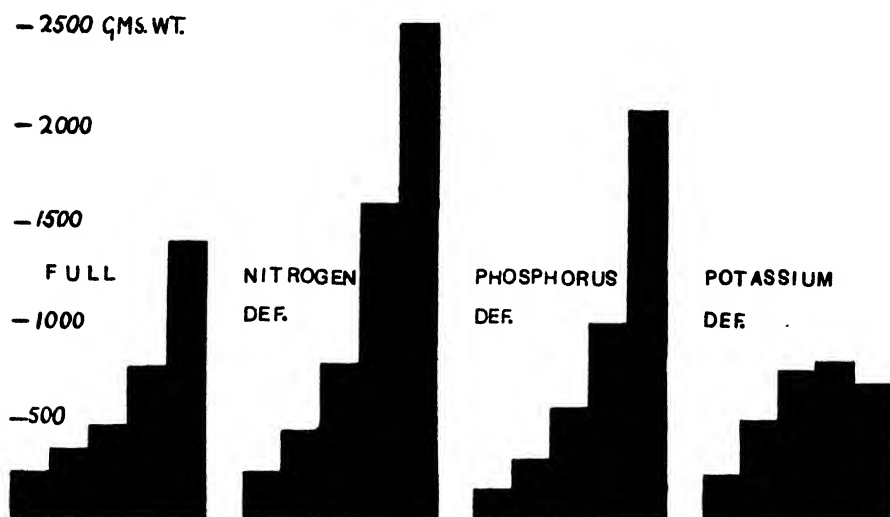


FIG. 1. The strength of succeeding internodes from above downwards.

the internode above and from the leaf attached at its base. The relative amounts of the tissues present in the stem were found by selecting at random two or three of the sections available for each internode; from these, drawings were made under a camera lucida and a low power of the microscope. The sections were stained with phloroglucin and hydrochloric acid, and the outline of the following four zones traced: (a) sclerenchyma, (b) lignified parenchyma, (c) unlignified parenchyma, (d) the central lacuna. The epidermis and islets of chlorophyllous tissue were included in the sclerenchyma zone, while the lignified vascular elements in the unlignified parenchyma were neglected.

It was found that considerable variation occurred in the depth of colour produced by phloroglucin, all tissues showing a pink coloration with phloroglucin and hydrochloric acid were accepted as lignified; it cannot be assumed, however, that the lignified tissues had all undergone lignification to the same extent. Nitrogen-deficient and phosphate-deficient plants alike showed lignification equal to that of fully-manured plants. Sections from potassium-deficient plants show considerably less depth of staining. All transitions were found from sections in which the sclerenchyma and vascular bundles were alone stained to sections indistinguishable

from those of fully-manured plants. The areas of the tissues were found with a planimeter, and the mean areas of the stem as a whole, of the three tissues, and of the central cavity in each internode were calculated. The use of mean values was considered permissible owing to the fact that variation between sections from the same internode is considerably less than that between sections from different internodes.

It was noted that even in cases of extreme reduction of the amount of tissue lignified in the potassium-starved plants, the vessels of the vascular bundles were always fully lignified.

Lignification consists of the incrustation and infiltration of the original cellulose cell-wall resulting in great changes in its physical properties. The unaltered cellulose wall is very elastic, but a comparatively small stress is sufficient to produce permanent distortion, but considerable extension can take place before fracture occurs. After lignification the tissue is far less extensible, and is capable of withstanding much greater stresses, but beyond the elastic limit fractures readily.

The thickness of the three zones under consideration appears to be markedly affected by the type of manuring. In Fig. 2 the thickness of sclerenchyma in each internode is shown graphically. It increases rapidly towards the base of the stem, but this increase is not proportionate to the increase in strength. For example, the strength of the fifth internode of the fully-manured plants is more than six times that of the first, whereas the thickness of sclerenchyma is only 2.4 times. The increasing strength in successive internodes from the apex cannot therefore be entirely ascribed to increase in thickness of sclerenchyma, and as, moreover, the lignified parenchyma shows only a slight increase in development, it would appear that the strength of these tissues must vary in the different internodes. The four diagrams in Fig. 2 show the same general form, but there are suggestive increases in the lower internodes of the phosphate- and nitrate-starved plants. These differences are not, however, significant. Reference to Fig. 3 shows that the thickness of the lignified parenchyma is much more uniform in all the internodes. Only in the case of the nitrogen-starved plants does the thickness increase steadily down the stem. There is, however, a striking reduction of lignified parenchyma in the potassium-starved plants. This failure to lignify the parenchyma results in a supernormal thickness of unligified parenchyma in the upper internodes as shown in Fig. 4. The large increase in the amount of unligified parenchyma is probably a partial explanation of the high value of the water-content of potassium-starved plants when compared with fully-manured plants. The almost constant breadth of the zone of the unligified parenchyma throughout the stem contrasts sharply with the remaining three types. The thickness is *significantly* increased above corresponding internodes of the fully manured in all the internodes of nitrogen-starved plants, in the third and

fourth internodes of the phosphate-starved plants and in all but the fifth of the potassium series.

The difference between the external and internal radii of the stem, i.e. the total thickness of the wall of the cylinder, is shown in Fig. 5. The

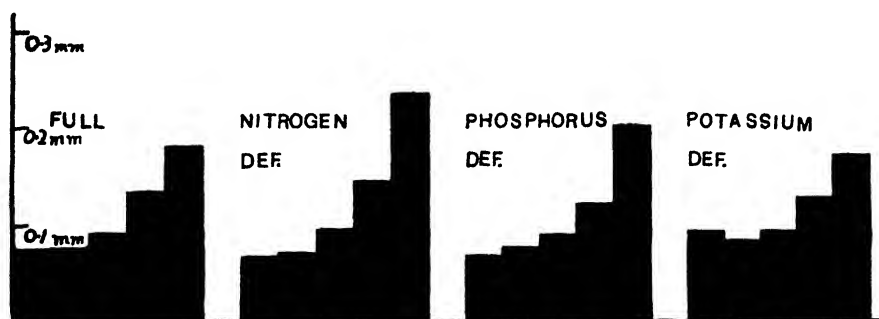


FIG. 2. The thickness of sclerenchyma in succeeding internodes from above downwards.

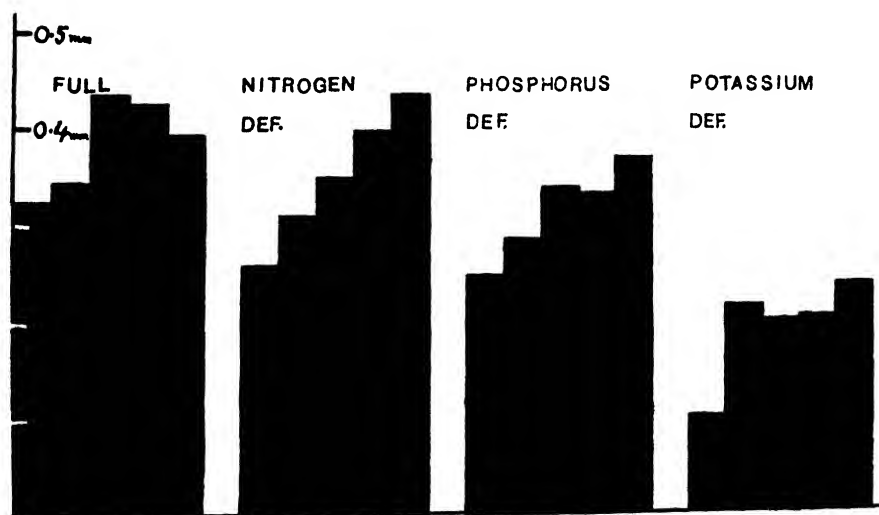


FIG. 3. The thickness of lignified parenchyma in succeeding internodes from above downwards.

thickness increases rapidly in the lower internodes; this is markedly affected by the manurial treatment. Nitrogen deficiency results in an increase in the thickness, a similar effect but not so clearly marked is to be observed in the case of phosphorus deficiency. With potassium deficiency the thickness is remarkably constant. Inasmuch as the diameter of the stem is in all cases reduced by mineral deficiency, a reduction in the diameter of the central cavity usually follows.



It was found that the ratio of the external diameter of the lignified tissue (i. e. the total diameter of the stem) to the internal diameter of the

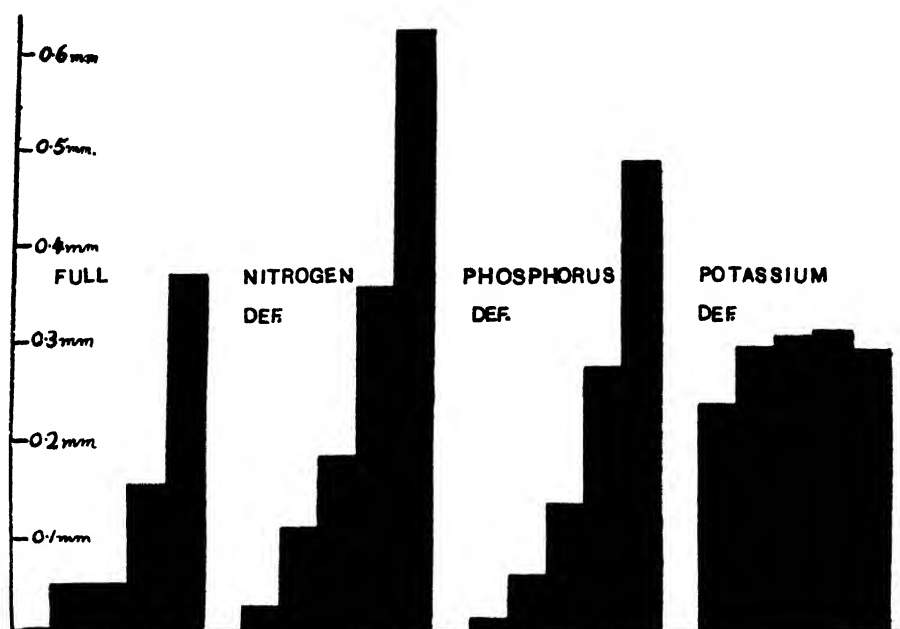


FIG. 4. The thickness of unligified parenchyma in succeeding internodes from above downwards.

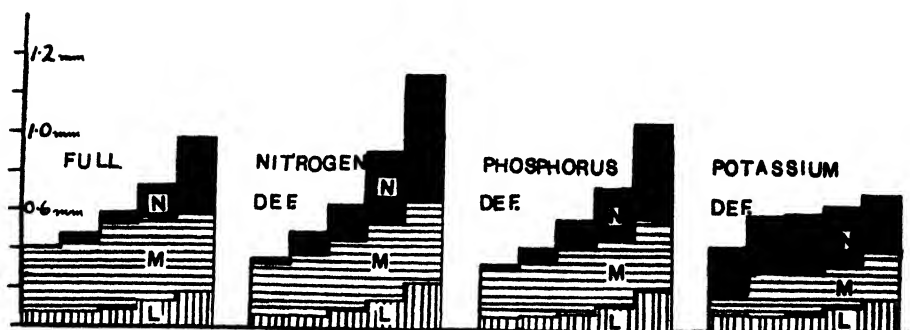


FIG. 5. The thickness of total tissue in succeeding internodes from above downwards, showing the relative proportions of the three tissues: L, sclerenchyma; M, lignified parenchyma; N, unligified parenchyma.

*lignified* tissue (sclerenchyma + lignified parenchyma) was constant for corresponding internodes with different manuring. The mean values for successive internodes are given in Table II, as well as the values for each manurial type.

TABLE II.

Internode number . . . . .	1.	2.	3.	4.	5.
Fully manured . . . . .	1·220	1·247	1·285	1·336	1·413
Nitrogen deficient . . . . .	1·214	1·237	1·289	1·375	1·435
Phosphorus deficient . . . . .	1·217	1·230	1·267	1·312	1·482
Potassium deficient . . . . .	1·152	1·249	1·294	1·388	1·522
Mean . . . . .	1·201	1·241	1·284	1·353	1·463

The value of 1·522 obtained for the fifth internode of the potassium-deficient plants is the only value differing significantly from those obtained for the fully-manured series. An analysis of variance showed that there was no significant manurial effect.

Thus the breadth of tissue that becomes lignified is directly proportional to the diameter of the internode. The existence of such a relationship renders it improbable that the number of parenchymatous cells that become lignified is dependent upon the amount of wall-forming materials available at the moment. The mechanical function of the sclerenchyma cells would seem to be pre-determined from the time they are laid down, and it appears that this is true of the cells of the lignified parenchyma also. This question is discussed later.

#### THE RELATION BETWEEN STRENGTH AND THE BREADTH OF THE TISSUES PRESENT.

The question arises, to what extent are the observed effects of manuring upon stem strength due either to alterations in the amounts of the tissues present, or to variations in the material composing them? It is probable that both these influences are at work, but the two cannot be directly separated owing to the fact that the strengths of individual tissues could not be estimated by the method used.

The relation between the strength of a hollow cylinder and the thickness of its walls, when strength is measured by the force required to crush it radially, is very nearly linear provided the material composing its walls is constant. The direct total and partial correlations between strength and the individual tissues have therefore been calculated and are given in Table III.

TABLE III.

	r <sub>12</sub>	r <sub>13</sub>	r <sub>14</sub>	r <sub>12·34</sub>	r <sub>13·24</sub>	r <sub>14·23</sub>
Fully manured . . . . .	0·854	0·340	0·945	0·485	0·246	0·825
Nitrogen deficient . . . . .	0·912	0·654	0·910	0·234	0·813	0·909
Phosphorus deficient . . . . .	0·740	0·563	0·859	0·410	0·486	0·644
Potassium deficient . . . . .	0·235	0·478	0·074	0·053	0·611	0·471

1 = strength. 2 = thickness of sclerenchyma. 3 = thickness of lignified parenchyma.  
4 = thickness of unlignified parenchyma.

With the exception of the potassium-deficient series, highly significant and positive total correlations are seen in all cases. The elimination of the effect due to variations in thickness of the other two tissues results in a decided reduction of the correlation between strength and sclerenchyma. Thus the anomalous result is obtained of low values of the partial correlation between strength and sclerenchyma and high values for that between strength and unligified parenchyma. Reference to Fig. 5 shows that, of the three tissues, unligified parenchyma is by far the most variable. Furthermore, the correlation between thickness of sclerenchyma and of unligified parenchyma is high (except in the potassium-deficient series), as is shown in Table IV.

TABLE IV.

Fully manured . . .	+0.772
Nitrogen deficient . . .	+0.823
Phosphorus deficient . . .	+0.815
Potassium deficient . . .	+0.015

The high correlation between thickness of sclerenchyma and of unligified parenchyma suggests that other factors may influence the strength. One of these is the morphological status of the internode as is shown by the high positive values of the correlations, internode number and strength (*a*), and internode number and total tissue thickness (*b*), as show in Table V.

TABLE V.

	( <i>a</i> )	( <i>b</i> ).
Fully manured . . .	+0.887	+0.878
Nitrogen deficient . . .	+0.936	+0.881
Phosphorus deficient . . .	+0.829	+0.864

It should be borne in mind that a high positive correlation between a variant and internode number indicates a high *negative* correlation with time, since the internodes have been arbitrarily numbered from above downwards.

It thus appears that the high value of the correlation of strength and unligified parenchyma ("14 Table III) arises from the fact that the unligified parenchyma is the most variable component of the total thickness of tissue (Fig. 5), and this variation is in the same direction as the increase of strength with internode number. When, therefore, the effect of thickness of unligified parenchyma is eliminated in the partial correlations of Table III, the effect of internode number is largely eliminated at the same time, leading to the anomaly noted above.

In the potassium-deficient series the correlation between strength and unligified parenchyma is greatly increased by the elimination of variance due to unligified parenchyma and to sclerenchyma. This is explained by

the value of the *negative* correlation between strength and sclerenchyma when age is eliminated, viz., 0.406 (significance of 100:1).

Attempts were made to obtain regression equations connecting strength, age, and the thickness of the mechanical tissues. With none of these was a good fit obtained. The equation  $S = (f_1)l + (f_2)m + (f_3)n + C$  where  $l$ ,  $m$ , and  $n$  are the thicknesses of the three tissues gave a good fit, but owing to the correlations of the tissue among themselves, the functions were difficult to interpret. It was observed, however, that the curve obtained when the mean strength of each internode was plotted against internode number did not differ significantly from an exponential curve in any case except in that of the potassium-deficient series. The linear regression equation between  $\log_{10}S$  and internode number was therefore calculated. The equations are:

Fully manured:	$\text{Log}_{10} S = 0.19116x + 2.1433$
Nitrogen deficient:	$\text{Log}_{10} S = 0.22603x + 2.22143$
Phosphorus deficient:	$\text{Log}_{10} S = 0.27430x + 1.91061$

In Table VI the calculated and observed mean values of  $S$  for each internode are given.

TABLE VI.

Internode.	Fully Manured.		Nitrogen Deficient.		Phosphorus Deficient	
	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.
1	229	216	241	280	151	153
2	360	335	445	472	306	288
3	465	521	786	793	572	541
4	771	809	1,604	1,335	1,000	1,018
5	1,407	1,256	2,543	2,247	2,097	1,915

The fact that the curve under discussion is an exponential curve demonstrates the fact that, within the limits of the error of the experiment, the strength of each internode bears a constant relation to the strength of the previous internode. The anti-logarithm of the coefficient of  $x$  in the above equation gives the relationship between strength of each internode and that of the internode next below it. These values, which may be said to characterize the effects of the manurial treatments on strength of straw, are as follows:

Fully manured	1.553
Nitrogen deficient	1.683
Phosphorus deficient	1.881

No rational expression was found that fitted the strengths found in the potassium-deficient series.

## DISCUSSION.

The foregoing data provide the results upon which is based the general conclusion that the strength of the stem is decreased in the potassium-deficient series and increased in the nitrogen- and phosphorus-deficient series, and that these differences from the normal decrease as the stem is ascended.

The dependence of carbohydrate supply upon potassium content offers a possible explanation of these observations. Potassium deficiency has no influence upon the number of young tillers produced, whereas both nitrogen and phosphorus deficiency cause a considerable reduction in their numbers. The two latter series have available, therefore, a larger amount of potassium per plant organ than the fully-manured series. If potassium plays an essential part in the mechanical properties of the tissues or in their formation, this provides a possible explanation of those results. The continued decrease in the strength of succeeding internodes suggests the operation of some deficiency factor which increases with time. Extensive work upon the nutrition of barley plants in this Institute has led to the conclusion that growth proceeds until the concentration of salts within the plant has fallen to a definite level. This is of interest in view of the tendency of the upper internodes of all four series to approximate to the same strength. Gregory and Richards (5) have shown that the assimilation rate of potassium-starved plants shows a marked recovery to a supernormal rate after its initial fall, and suggest that this is connected with the supply of additional potassium from dying leaves and tillers. It is possible that some such recovery superimposed upon the age drift of strength accounts for the recovery to a supernormal value of the strength in the second and third internodes.

It has been demonstrated that there is a 'position' effect upon the strength of the tissues, the intrinsic strength as well as the thickness of the tissue increasing as the stem is descended. The change in the mechanical properties of the tissues is shown by the highly significant values found for the correlation between internode number and the ratio strength : thickness of total tissue. These are :

Fully manured	+0.867
Nitrogen deficient	+0.938
Phosphorus deficient	+0.729
Potassium deficient	+0.491

The decrease in strength of succeeding internodes in time may therefore be ascribed firstly to a decrease in the efficiency of the mechanical tissues and, secondly, to decrease in their amount.

It has also been shown that within the limits of experimental error the ratio of the external radius to the internal radius of the mechanical tissue is

constant for internodes of the same morphological age and is independent of manuring, except in the case of the lowest internode of the potassium-deficient series. This is of interest in view of the fact that the stems are laid down in embryonic form very early in the history of the plant, before internal manurial deficiency has occurred, except in the case of potassium-starved plants in which the incidence of internal starvation as evidenced by the sharp fall of assimilation rate is very early (5).

If cell size is proportional to the radius of the internode, the total number of mechanical cells in a cross-section of any internode of the same morphological status must be constant. This would indicate that the mechanical tissues are composed of elements whose function is determined irrespective of manurial deficiency. On the other hand, their mechanical strength is affected by the type of manuring given, the evidence pointing both to an effect upon the thickness of the tissue and upon its mechanical efficiency. The latter may be due either to alteration in the structure of the cell-wall or in its composition.

#### SUMMARY.

The paper deals with the results of an investigation of the effect of manurial deficiency upon the strength and anatomical structure of barley straw. The force in grm. weight required to crush 1 cm. length of stem radially is taken as a measure of strength.

The strength of succeeding internodes of fully-manured plants falls off rapidly. Nitrogen and phosphorus deficiency results in a large increase in the strength of the lower internodes, while potassium starvation decreases the strength of the lower and increases that of the middle internodes. The effects of manurial deficiency are most marked in the lower internodes, the upper ones approximating to the normal.

The variation in the thickness of the mechanical tissues follows that of strength but is not sufficient to account for the large differences observed. Total and partial linear correlations between strength and the morphological status of the internode, and between strength and the thickness of the three tissues, sclerenchyma, lignified parenchyma, and unlignified parenchyma, have been calculated. The conclusion is reached that the observed fall in strength of succeeding internodes is due both to decrease in the efficiency of the mechanical tissues and also to decrease in their actual amount.

Equations connecting strength and the morphological status of the internode are found to give a good fit in the fully manured, nitrogen-deficient and phosphate-deficient series. The relation between strength and internode number is logarithmic, the strength of each internode being a constant fraction of that next below, within the limits of the error of the experiment, the value of the fraction being dependent on the type of manuring applied.

The ratio of the external radius to the internal radius of the mechanical tissues is found to be constant for internodes of the same status independent of manuring. It appears that the mechanical function of the elements composing them is determined at a very early stage.

It is suggested that the observed effects of mineral deficiency are explicable on the assumption that potassium is essential to the production of an efficient mechanical tissue.

The work reported here was carried out at Rothamsted at the suggestion of Dr. F. G. Gregory, and the author's thanks are due to Sir John Russell for granting the necessary facilities. The author desires to thank Professor V. H. Blackman and Dr. F. G. Gregory for stimulating advice and criticism.

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# Studies of the Physiological Importance of the Mineral Elements in Plants.

## I. The Relation of Potassium to the Properties and Functions of the Leaf.

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With six Figures in the Text.

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### I. INTRODUCTION.

IT has long been known that an adequate supply of potassium is necessary for the photosynthesis of starch, and since the element has not been found to occur in starch or any other important product of carbon assimilation, there has been much speculation concerning its part in the process. That its role is important is shown by the wide range of plants in which its presence is known to be necessary. Starch formation has been shown to be facilitated by potassium in such diverse cases as the algae, the protenemata of mosses (Reed, 16), sunflower seedlings (Briggs, 5), and the potato plant (Maskell, 14).



The last species affords peculiar advantages for the study of this problem for two reasons. As is well known to all growers, its potassium requirements are unusually high, in correlation, perhaps, with its exaggerated formation of starch. This is likely to help the experimental handling of the problem, especially under field conditions, where delicate control cannot be established, and also when quantitative analyses are required. In the second place, the plant segregates its abundant reserve carbohydrate in well-defined storage tubers, thus enabling it to be estimated with ease.

Taking advantage of these facts, Maskell (14) has made an investigation with potato plants growing under normal agricultural conditions. The only unusual manipulation was the approximate control of the potash content of the soil, which was varied on neighbouring plots. On land supplied with potassium in the form of sulphate, the quantity of starch formed per acre was in each case higher than when no potassium was added. This was partly accounted for by an increased weight of tubers, and partly by a larger percentage of starch in the dried material. Maskell found that correlated with this greater starch formation there was, with potassium sulphate, a rise both in the rate of starch formation in the leaves, and in the rate of its translocation from them. There remains the problem of how these accelerations are brought about. To help in solving this it seemed desirable to discover what further effects, if any, potassium has upon the functions of the leaf, and during the summer of 1926 a number of observations were carried out towards this end. Data were collected bearing upon the starch formation, translocation, number, area, weight, water content, and senescence of potato leaves.

## 2. LEAF NUMBER.

Other things being equal, an increase of leaf area should lead to an increase in the total quantity of starch formed by any individual plant. It is therefore of interest to discover whether additions of available potassium lead to such an increase of surface, but it is unfortunately very difficult with the potato plant to obtain a reliable estimate of the leaf area. A normal adult plant of the available variety (Kerr's Pink) may produce as many as two hundred leaves, with an average of seven leaflets each. It is, moreover, necessary in comparison between two sets of plants to take a large number of individuals, since variation is high.

In the season 1927 an attempt was made, with the variety Arran Comrade, which produces a less luxuriant growth than Kerr's Pink, to estimate the average leaf area of plants receiving high and low supplies of available potassium. Weekly samples were taken from the time the leaves appeared above the ground, both the high and low potassium samples containing ten plants, one being selected at random from each of ten plots.

All leaflets were stripped from the plants taken, and photographed on blue print paper as quickly as possible. The prints were planimetricd at leisure, and the total areas summed. It was proposed to continue this sampling throughout the life-cycle of the plant, but after the first few samples the number of leaves had become so great that the photographic printing could not be carried out with the assistance available, and the experiment had to be brought to a close. Up to this point the results indicated were as follows:

TABLE I.

*Average Leaf Area of Ten Potato Plants in sq. cm.*

	Available Potassium.	
	Low.	High.
July 6	30.01	67.96
13	71.62	281.93
20	385.92	1217.25

An experiment carried out on August 3 with five plants in each sample indicated a reversal in the relative areas, which was accompanied by a corresponding reversal in dry weights. Thereafter dry weight measurements, which were continued throughout the growing period, indicated very slight differences between the two groups, which were not statistically significant, since variation was high and the samples small.

In the absence of reliable results from direct measurements a second method is of interest. An approximate indication of leaf area, when dealing with a reasonably large sample, is afforded by the total number of leaves. This crude estimate may be improved by measuring the area of a selected leaf or leaflet. Knowing the average number of leaves produced, and the average area of a selected leaflet, some idea may be formed of the relative leaf areas of the plants on different plots. Measurements of this kind were carried out on adult material (var. Kerr's Pink) in the season 1926.

TABLE II.

*Plot totals: Number of Leaves on Twenty Plants.*

		No Potassium added.	Potash Manure Salts.	Potassium Chloride.	Potassium Sulphate.
Series 1.	Aug. 12	2419	2023	2305	1980
Series 2.	Aug. 15	2231	1965	2488	2543
Series 3.	Aug. 18	3098	2193	2606	2748
Treatment Totals		7748	6181	7399	7271
Mean		2582.67	2060.33	2466.33	2423.67

To estimate the leaf number, observations were made on 1/5<sup>th</sup> acre plots, each containing about 300 plants. Twenty plants were taken at

random on each plot, and the total number of leaves, irrespective of their condition, counted on each plant. Four series of counts were made, each involving four plots. In the first three series, the plots had received respective dressings of potassium chloride, potassium sulphate, 'potash manure salts' (a low-grade fertilizer), and no additional potassium, besides an otherwise normal potato manuring. The results of these counts are summarized in Table II.

There appeared to be a reduction in the number of leaves produced by plants receiving additional potassium, and the figures were therefore examined by Fisher's Analysis of Variance method (11). It was assumed that the difference of occasion had no effect upon the leaf number, since the dates were not far removed from one another. The variance due to individual plot effects could then be separated from the treatment effects, and the following values were obtained :

TABLE III.

*Analysis of Variance of Leaf Number.*

	Sums of Squares.	Degrees of Freedom.	Variance.	Log <sub>e</sub> Variance.	Z Found.	For $P = 0.05$ .
Treatment	457736	3	152579	11.9355	0.7798	1.0953
Plot	494954	2	247477	12.4193		
Remainder	311431	6	51905	10.8572		
Total	1264121	11				

The 'remainder variance' represents the variance due to differential responses of the various plots to different manurial treatments. The variance in leaf number due to treatment is greater than this remainder variance, and it is necessary to ascertain whether the excess could arise from any chance combination of manures and plots, or whether it is great enough to indicate a significant effect of treatment upon leaf number independent of the differences in plots. A result is considered significant when it could not arise by chance combinations more than once in twenty times ( $P = 0.05$ ). The criterion developed by Fisher for this comparison is half the difference between the natural logarithms of the two variances ( $= Z$ ). The value of  $Z$  required for significance is dependent upon the number of degrees of freedom available for estimating the variances, and in the present instance is  $= 1.0953$ . The value  $\frac{1}{2} (11.9355 - 10.8572) = 0.7798$ , and therefore fails to reach significance.

The number of leaves counted on the P.M.S. plots was considerably lower than on the others, and although in the general analysis this difference is obscured by the similarity between other treatments, there remains the possibility that the mean P.M.S. value is significantly lower than, say, the mean value for no additional potassium. The difference between these

mean values was therefore examined by means of their standard error ( $S$ ).

As an estimate of this quantity the value  $\sqrt{\frac{\text{Remainder variance} \times 2}{\text{number of replications}}}$  was taken, and since the available samples were small the further quantity

$$t = \frac{\text{P.M.S. mean} - \text{no K mean}}{S}$$

was also determined. The value of  $t$  required for any given degree of probability is dependent upon the number of degrees of freedom ( $n$ ) available for its estimation. Tables of  $t$  for varying probabilities and values of  $n$  are given by Fisher (11). For  $P = 0.05$  with 6 degrees of freedom the value is 2.447, and as calculated above the several comparisons give:

No K — P.M.S. 2.805; KCl — P.M.S. 2.183;  $K_2SO_4$  — P.M.S. 1.952. The average number of leaves produced on P.M.S. plots is therefore significantly lower than on plots without added potassium, but there is no significant difference between the means when P.M.S. is compared with other treatments. Neither are the differences between the addition and absence of KCl or  $K_2SO_4$  significant since they are even less than that of the previous comparisons.

In the fourth series of counts the case of  $K_2SO_4$  was examined further. A different arrangement of plots was employed, in which the sulphate was withheld or added to the extent of 1, 2, or 4 cwt. per acre. Counts were carried out on each of the four plots in the manner described for series 1-3. The total number of leaves on each plot, twenty plants being counted, were as follows:

TABLE IV.

*Series 4.*

Potassium sulphate added in cwt. per acre.	Leaves counted.
0	2,124
1	2,017
2	1,682
4	1,760

*Analysis of Variance of Leaf Number.*

	Sums of Squares.	Degrees of Freedom.	Variance.	Log <sub>e</sub> Variance.	Found. $Z$	For $P = 0.05$ .
Treatment	6545.8	3	2181.9	7.6881	0.3663	0.4787
Remainder	79797.6	76	1049.9	6.9566		
Total	86343.4	79				

In the comparison between treatment and remainder variances the significant value of  $Z$  is here 0.4787. Half the difference between the

natural logarithms as calculated = 0.3663, and the treatment variance is therefore not great enough to be significant. An inspection of the values at the head of Table IV will show, however, that there remains the possibility of a significant difference between 'high' and 'low' treatments (2 and 4 compared with 0 and 1).

Treatment variance may therefore be further analysed as follows:

TABLE V.

	Sums of Squares.	Degrees of Freedom.	Variance.	Log <sub>e</sub> Variance.	Z Found.	Z For $P = 0.05$
High—(2 and 4) with low— (0 and 1)	6107.5	1	6107.5	8.7174	0.8804	0.6729
Other com- parisons	438.3	2	219.2			

For a comparison between these variances and the remainder variance the significant limit of  $Z = 0.6729$ . Nearly all the treatment variance is accounted for by the comparison between high and low treatments which gives a value for  $Z$  of 0.8804. The plots receiving high potassium sulphate thus show a significant reduction in the number of leaves produced, when compared with little or no addition.

### 3. THE AREA OF A SELECTED LEAFLET.

The leaflet chosen for this estimation was the penultimate pinna of the fourth leaf counting from the apex of any haulm. No differentiation was made between left and right pinnae, but to avoid the correlation known to exist between opposite pinnae of the same leaf (14) samples were taken singly and never in opposite pairs. The material to be measured was obtained from a field experiment of sixteen plots, twelve of which were also sampled in making the leaf counts of series 1-3. The experiment contained four treatments, each repeated four times. The plots were arranged as a four by four 'latin square', each treatment occurring once, and only once, in each row across, and in each column up and down the experiment. With this limitation they were placed at random within the square. The treatments, as previously stated, were, no additional potassium, potassium chloride, potassium sulphate, and potash manure salts.

Leaflets were collected for measurement every fourth day from July 12, when the plants had already developed a large number of leaves, to September 10, when the leaflets were turning yellow. Each sample consisted of six leaflets per plot taken from different plants at random.

A rack containing sixteen stoppered weighing bottles was taken on to the field, and each sample of leaves enclosed, as it was picked, in a bottle numbered to correspond with the plot. The small quantity of air enclosed

by the bottles was soon saturated with water vapour and further transpiration by the leaflets thus prevented. This was important, as potato leaves shrink markedly with loss of moisture (see p. 183). When sampling was completed the rack was taken to a field laboratory, where the leaflets were

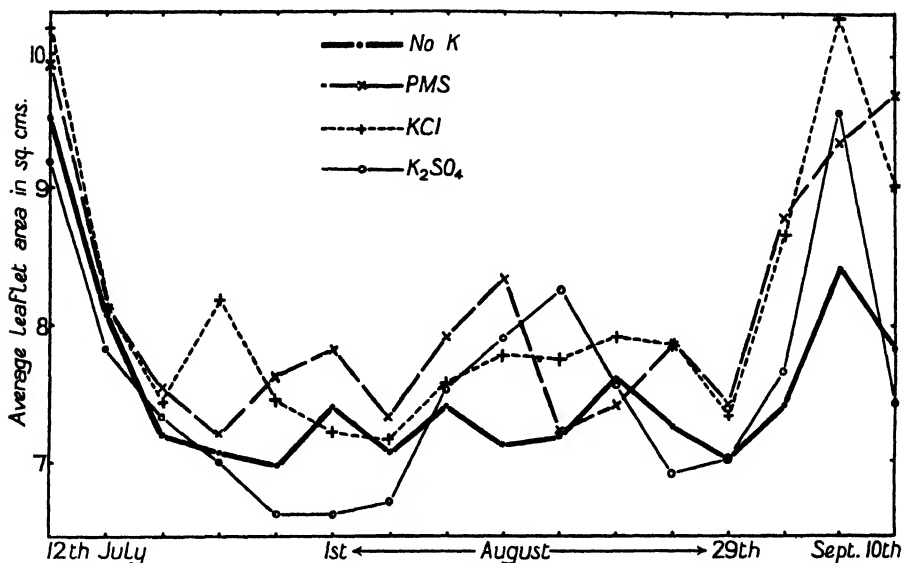


FIG. 1.

taken from the bottles and photographed on blue print paper, and the areas of the prints afterwards measured with a planimeter. The mean treatment values of the twenty-four leaflets on each occasion are shown in Fig. 1.

An analysis of the figures gave the following results:

TABLE VI.

*Analysis of Variance of Leaf Area.*

	Sums of Squares.	Degrees of Freedom.	Variance.	Log <sub>e</sub> Variance.	Found. $Z$	For $P = 0.05$ .
Treatment	17.90187	3	5.96729	1.7863	1.0146	0.7798
Plot { Rows	29.04102	3	9.68034			
Plot { Columns	1.37800	3	0.45933			
Remainder	4.70660	6	0.78443	1.7571		
Occasion	153.39477	15	10.22632	2.3253	1.2841	
Differentials	284.40521	225				
Total	490.82747	255				

Owing to the planning of the experiment it is possible to compare similar plots both across and up and down the area. The variation between like plots in the transverse direction is given in the above analysis by 'Rows' and the variation travelling at right angles to this by 'Columns'. The two

added together give the total variance ascribable to plot differences. The remainder variance is the random variance due to chance combinations of plots and treatments without reference to occasion, and that labelled 'differential', the third degree differential variance due to chance combinations of occasion, plot, and treatment. As a basis for comparison in tests of significance the 'remainder' variance 0.78443 is used. The values of

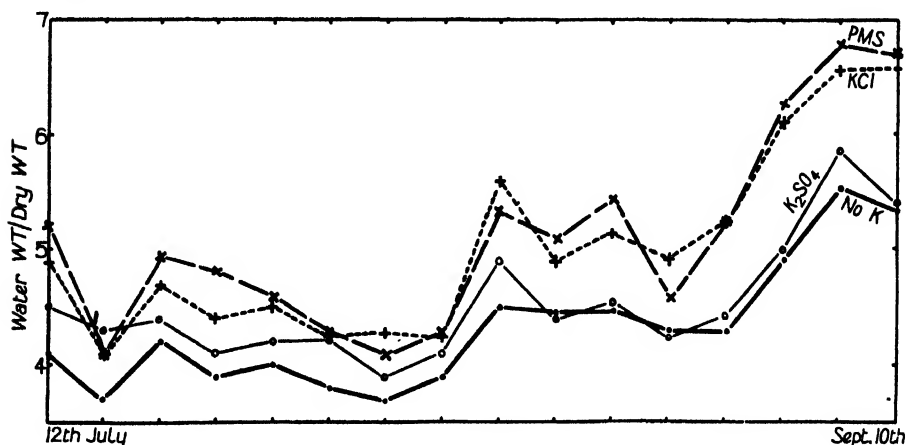


FIG. 2.

$Z$  found and the values required for significance ( $P = 0.05$ ) are given in Table VI, and show that the variance due to treatment, rows, and occasion are all significant. The columns variance, however, is not significant.

The mean areas of leaflets given by the different treatments were:

No potassium 7.590;  $K_2SO_4$  7.578; KCl 8.112; and P.M.S. 8.124 sq. cm. It is, therefore, evident that the treatment variance is almost entirely accounted for by the comparison between no added potassium +  $K_2SO_4$  on the one hand, and KCl + P.M.S. on the other. There is practically no difference between  $K_2SO_4$  and absence of added potassium. The KCl and potash manure salts are alike in containing a rather high percentage of chlorine, which is absent in the other treatments. The correct interpretation, therefore, of the significant treatment variance is, probably, to ascribe the increase in area which it denotes to the action of chlorine rather than that of potassium. This view receives further support from the results of the following section which suggest a possible mechanism for the changes.

#### 4. WATER CONTENT.

It was shown by de Vries (10) that potassium is associated with organic acids in the cell sap of various tissues, including leaves, and he suggested that the power of the vacuoles to absorb water is due in part to the dissolved

potassium. With a view to investigating the problem in its relation to leaves, the water content of a large number of leaflets was examined. The material was that also used in leaf area measurements, the collection and initial handling of which has already been described on p. 178. Immediately on arriving at the field laboratory the leaflets were weighed in their respective bottles without further handling. No attempt was made to secure individual weighings of the leaflets, as these were not wanted. After being photographed for the area determinations, the leaflets were returned to the weighing bottles and put to dry in an oven at approximately 100° C. After twenty-four hours the bottles were transferred to a desiccator and, when cold, stoppered and weighed. The difference between the first and final weighings was taken as the total quantity of water in the leaflets. The moisture content of the leaves was expressed as water weight / dry weight (see Fig. 2), and the variance of these results analysed by the method already employed, the following values being obtained :

TABLE VII.

*Analysis of Variance of Water Content per Lot of Six Leaflets.*

	Sums of Squares.	Degrees of Freedom.	Variance.	Log <sub>e</sub> Variance.	Z Found.	For $P = 0.05$ .
Treatment	29.848792	3	9.949597	2.2975	1.8240	0.7798
Plot { Rows	1.312332	3	0.437444			
Columns	0.168023	3	0.056008			
Remainder	1.554752	6	0.259125	2.6495		
Occasion	110.827712	15	7.388514	1.9999	1.6752	0.6931—0.6729
Differentials	23.861432	225				
Totals	167.573043	255				

The treatment variance is clearly significant. The weighing for the individual treatments gave the following average values :

No potassium 4.328 ; P.M.S. 5.139 ; KCl 5.035 ; K<sub>2</sub>SO<sub>4</sub> 4.509.

These figures fall readily into two groups ; P.M.S. and potassium chloride treatments giving high water contents ; no potassium and potassium sulphate giving relatively low water contents. The first two are alike in containing chlorine, and the total treatment variance can therefore be further analysed.

TABLE VIII.

	Sums of Squares.	Degrees of Freedom.	Variance
Cl—no Cl . . . . .	28.4755	1	28.4755
Other comparisons . . . . .	1.3733	2	0.6767
All treatments as in Table 7	29.8488	3	

Practically the entire treatment variance is therefore due to the chlorine comparison, and there is no possibility of a significant potassium effect on



the water weight / dry weight ratio. The foregoing results, therefore, do nothing to support the view that the osmotic pressure in leaf cells is due to the presence of dissolved potassium.

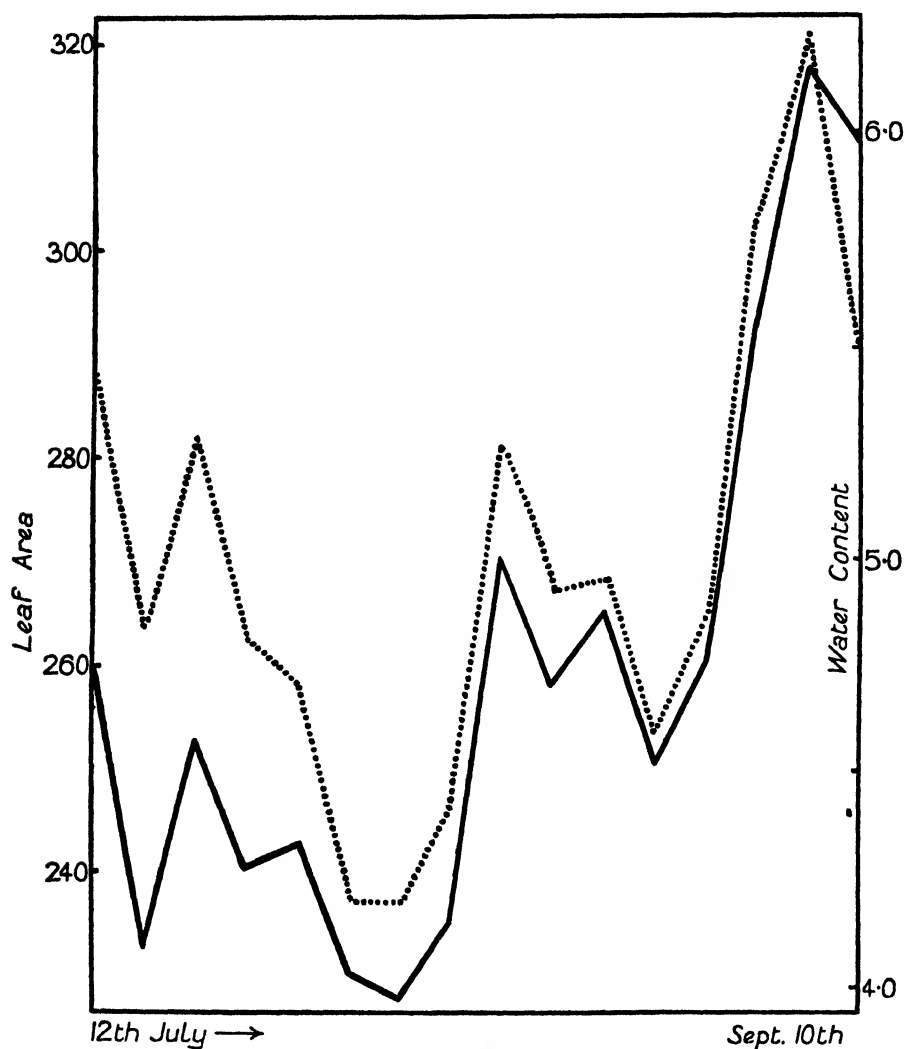


FIG. 3. Water content per gm. of dry weight is shown by the continuous line; leaf area (sq. cm.) per gm. of dry weight by the dotted line.

In addition to the treatment variance there was a large and significant variance due to 'occasion', the water content varying, that is to say, on the different sampling occasions, even on identical plots. These variations were no doubt dependent upon the complicated soil and atmospheric conditions which control transpiration, and a detailed analysis is beyond the scope of the present paper. An interesting correlation was observed, however,

between water content and area, both of which had been observed, on the same series of leaflets. To improve the comparison, areas were calculated, like the water contents, in terms of unit dry weight. A similar result might have been obtained by giving the weights of water in absolute quantities per leaflet. Fig. 3 indicates the very close nature of the correlation.

The fluctuations in leaf area cover as much as 25 per cent. of the mean value, and although changes of water content do not necessarily account for all this they must be the cause of a very high proportion. It follows, therefore, that water content, and the factors controlling it, must be taken into careful account in estimations of the leaf area of such species as the potato. In the foregoing analysis of leaf area measurements, these effects are included under 'occasion', and the estimate of treatment variance is therefore not disturbed by them. In experiments where periodic measurements of leaf area are used as an index of growth, the question seems to require more attention than it has received. Similar fluctuations of leaf area, depending upon external controlling conditions, have been observed with sunflower plants by Thoday (18).

#### 5. LEAF WEIGHT.

In the observations on leaf water content just recorded, incidental measurements were obtained of the weights of the leaflets after drying. As these weights are also of interest in relation to other properties of the leaf, they were themselves examined by the analysis of variance method. The principal figures are given in the following table :

TABLE IX.

*Analysis of Variance of Dry Weight of Leaflets.*

	Sums of Squares.	Degrees of Freedom.	Variance.	Log <sub>10</sub> Variance.	Found. $Z$	For $P = 0.05$ .
Treatment	2339	3	779.7			
Plot { Rows	13458	3	4486.0	8.4088	0.8756	0.7798
{ Columns	845	3	281.7			
Remainder	4672	6	778.7	6.6577		
Occasion	29440	15	1962.6	7.5822	0.4623	0.69—0.67
Differentials	147554	225	655.8			
Total	198308	255				

In this analysis the variances due to treatment, columns, and differentials, are obviously not significant since they are actually less than the remainder variance or scarcely exceed it. Of the other two, 'occasion' also proves to be non-significant, indicating that the leaflets had attained much the same weight at whatever stage of the life-cycle they were sampled. There was not, for instance, any marked tendency for leaflets produced late in the season to show a more restricted growth than those produced early,

nor could one expect to find a correlation between their development and the weather. The large time variance in area shown in Table VI cannot, moreover, be accounted for by variations in the quantity of dry material, and its dependence on water content is thus confirmed.

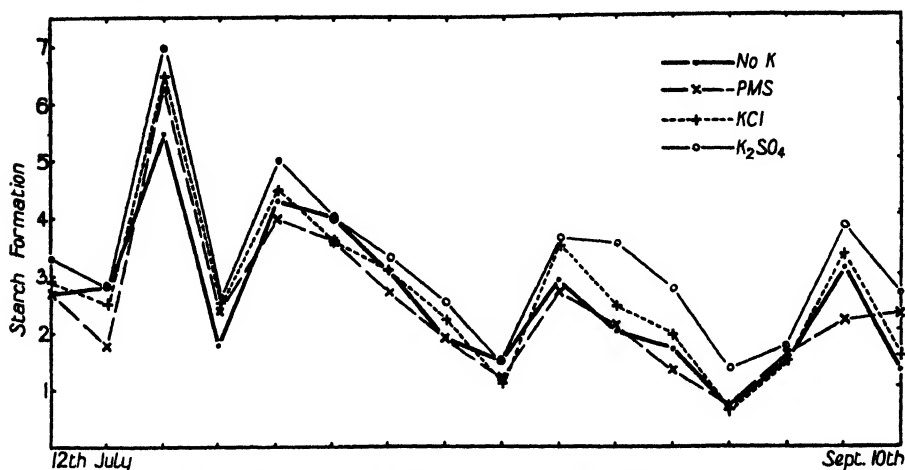


FIG. 4. Starch content, in terms of tone scale numbers, for 4-day periods, from July 12 to Sept. 10.

## 6. STARCH FORMATION AND TRANSLOCATION.

Reference has already been made (p. 174) to the observations of Maskell (14) on the effect of potassium manuring upon the rates of formation and removal of starch. His experiments were carried out in the summer of 1925 with the potato variety, Kerr's Pink. Four plots, receiving respectively no additional potassium, low grade potash manure salts (P.M.S.), potassium chloride, and potassium sulphate, were sampled on eleven occasions. An extension of this experiment was carried out by the present author in the following season. Instead of using single plots for each treatment, sampling was carried out over a four by four latin square, containing the same treatments, each treatment being replicated four times. The samples were taken every fourth day from an early stage in growth up to a period when yellowing was well advanced, covering sixteen occasions in all. The material was similar to that taken for leaf area and water content estimations, i. e. a penultimate pinna was removed from each of six leaves, the leaves being always the fourth counting from the apex; the plants and haulms from which they were derived were taken at random from the plot. Only green leaflets of healthy appearance were taken, the experiment being stopped when leaflets at the selected position began to turn yellow.

The method of estimating starch formation was that employed by Maskell, with minor alterations. The selected leaflets, together with their

opposite pinnae, were covered with black paper envelopes on the evening prior to their removal from the plant. At one o'clock on the following day one envelope of each pair was removed and the leaflet, by now destarched, was exposed for two hours. At three o'clock the leaflets were picked in pairs, decolorized with alcohol, and immersed in a strong solution of alcoholic iodine diluted with an equal quantity of water. The depth of the colour produced was judged in each case against a black-violet colour scale, Ridgway 59<sup>""</sup> (17). The scale contained nine tones, and, with practice, judgement could be made to half a tone. The difference between the tone numbers of the exposed and covered leaflets was taken as a measure of the starch formation during the standard two hours' exposure, and the mean of the six numbers as the representative value for the plot. The treatment values for each occasion are shown graphically in Fig. 4, and analysis of the results yielded the following data:

TABLE X.

*Analysis of Variance of Starch Formation.*

	Sums of Squares.	Degrees of Freedom.	Variance.	Log <sub>e</sub> Variance.	Found. $Z$	For $P = 0.05$ .
Treatment	20.798	3	6.933	1.9363	1.9441	0.7798
Plot { Rows	9.324	3	3.108	1.1339	1.5429	0.7798
Columns	1.799	3	0.600	1.4892	0.7206	0.7798
Remainder	0.840	6	0.142	2.0481		
Occasion	425.705	15	28.380	3.3457	2.6488	0.6931
Differentials:						
Time × Treatment	18.707	45	0.418	1.1277	0.5398	0.6499
Time × Rows	34.151	45				
Time × Columns	17.951	45				
remainder	23.588	90				
Totals	552.863	255				

The significant variances are shown by heavy type. A large variation is ascribable to treatment, and it is therefore of interest to discover to which of the comparisons it is due.

The mean values for starch formation were No K 2.55; P.M.S. 2.47; KCl 2.72; K<sub>2</sub>SO<sub>4</sub> 3.51; and it is therefore clear that the bulk of the treatment variation is accounted for by the comparisons between potassium sulphate and the remaining three treatments. To examine the significance of these differences the quantity

$$t = \frac{M_1 - M_2}{s}$$

was calculated as in the case of leaf number. The values obtained were K<sub>2</sub>SO<sub>4</sub>—No K 4.424; K<sub>2</sub>SO<sub>4</sub>—P.M.S. 4.729; K<sub>2</sub>SO<sub>4</sub>—KCl 3.640; KCl—No K 0.6383. Reference to Fisher's tables shows that as six degrees of freedom are available for the estimation of  $s$ , the differences

between sulphate and other treatments could arise by chance less than once in twenty times. The difference caused by adding KCl on the other hand, does not indicate a definite increase. The small decrease shown by the P.M.S. mean is certainly not significant, and the large treatment variance is, therefore, almost entirely due to the beneficial effect of potassium sulphate on the rate of starch formation.

The differential variances were further analysed. Interest centres upon the varying effects of treatment at different times, since if such a variance were found significant the data would be worth further investigation in regard to the correlation between manurial conditions on the one hand, and weather or ageing on the other. As shown in Table X, however, this variance (Time  $\times$  Treatment), just failed to reach a significant value. This may well be due to lack of differentiation between the treatments containing chlorides, masking the effects produced by the sulphate, and the curves of Fig. 4 do suggest that the effect of  $K_2SO_4$  is greatest in the later part of the season. The correlation between ageing and treatment is investigated further in the following section.

In this experiment the period during which the leaflets were darkened was so long that the starch was almost entirely removed from them. It was not possible, therefore, to analyse the tone numbers of the covered leaflets for a rate of removal of starch as was done by Maskell. This method is furthermore open to the objection that since the quantity of starch formed in light is dependent upon treatment, the amount remaining after a period of darkness need not be proportional to the rate of its removal, but will also be influenced by the different amounts initially present. Thus, since potassium sulphate increases the amount of starch formed during illumination, its effect, if any, in accelerating translocation will tend to be masked. As it was desirable, however, to obtain some information on this point, a special experiment was carried out midway through the season. Sampling was similar to that in the starch formation experiment. On each occasion six leaflets from the selected position on the plant were taken from each of the sixteen plots, and removed in a bottle, numbered to correspond with the plot. The leaflets were not covered or manipulated in any way before picking, so that on decolorizing and treating with iodine a measure was obtained of their starch content under natural conditions. Samples were taken at every third hour from 10 a.m. on July 27, to 10 a.m. the following day. On July 27 the sky was overcast and total darkness set in at 10 p.m., lasting until 4.15 next morning. During this period the collection of the leaflets was carried out with the help of an electric torch, the illumination from which could have no appreciable effect on the starch content of the leaflets. A number of leaves of the required position had previously been marked with white tabs to facilitate this work. After each collection the material was carried to a field

laboratory and immersed at once in alcohol, the starch determinations being carried out subsequently at leisure. Average values from twenty-four determinations were thus obtained for each treatment on each occasion. The results are shown graphically in Fig. 5. From 1 p.m. to 10 p.m. on

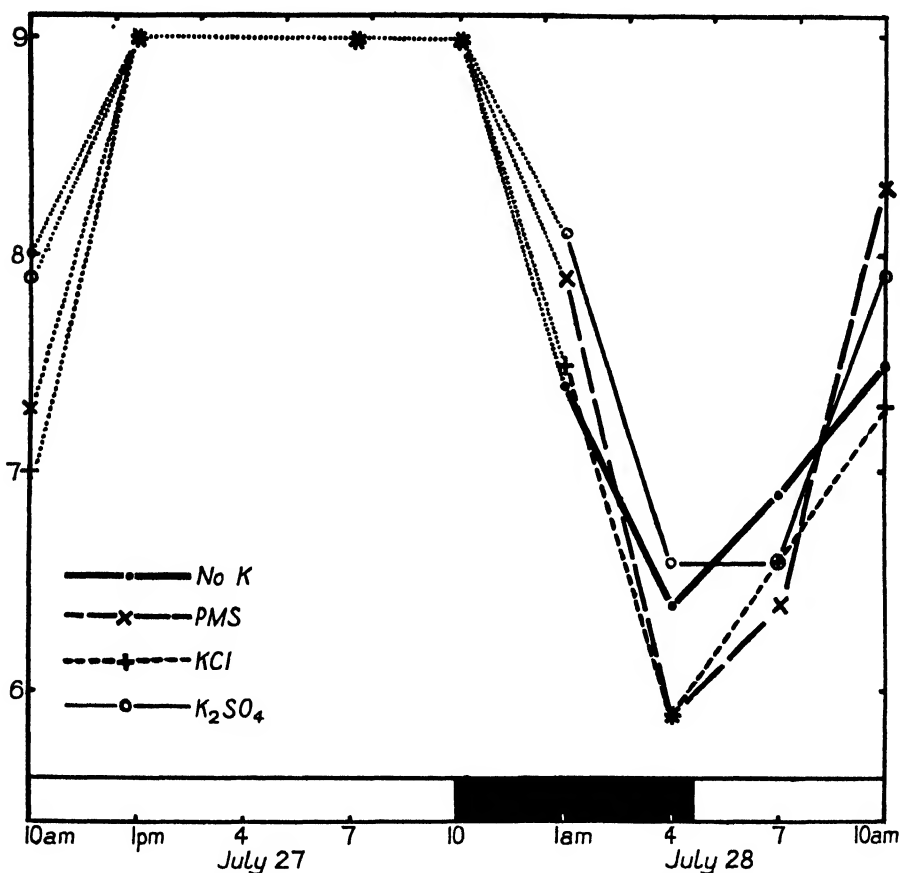


FIG. 5. Starch content, in terms of tone scale numbers, during 24 hours.

the 27th, the treated samples gave intense black colours, their starch contents being, in consequence, beyond the range of the determination. From 10 p.m. to 4 a.m. there was a steady reduction in the quantity of starch in the leaves of every treatment series, and from 4 a.m. onwards a rise. During the whole of the time from 10 p.m. until 10 a.m., during which the method could be usefully applied, distinct values were obtained for the different treatments. The most important point is that the leaflets receiving potassium treatments show a relatively high starch content in the afternoon and evening, and that during the night their curves cut that of the no potassium treatment, presumably owing to the faster rate at which starch is removed from them. The daytime samples, in which the colour was

invariably fully black, were discarded and the variance of the remaining values analysed to see whether the indicated differences between the treatments were significant. Owing to the relatively small number of sampling occasions, viz., five, the treatment variance failed to reach significance, and it could not, therefore, be expected that the time  $\times$  treatment differential variance, indicating differences in the rate of starch removal, would be significant. Neither was the largest of the differences between two treatment means great enough to indicate a certain result. Further analysis was therefore abandoned, and the results of the experiment must be regarded as suggestive rather than conclusive.

## 7. COLOUR.

As a first attempt to examine the effects of potassium upon ageing, the most obvious attribute of the leaf was considered. In the early part of July a change of colour first became apparent, at which time it was limited to the lower leaves, the middle and upper remaining a fresh green until a much later period. The original colour differed slightly on different plots, and a practised eye could judge the manurial treatment from the massed colour of the plot as seen from a small distance. On the experiment previously referred to, for instance, the P.M.S. plots had an obviously lighter tone than the potassium sulphate plots, and in an adjacent experiment involving nitrogenous manures, the colour differences were much more obvious, the presence of abundant nitrogen being correlated with a lush greenness.

The latter series of plots, referred to in future as the 'Quantitative Experiment' was of the 'window pane' variety. It contained four equal blocks, each of which had sixteen plots within it. The treatments it was designed to investigate were increasing doses of potassium and ammonium sulphates. Application was made at the rate of 0, 1, 2, or 4 cwt. per acre, and there was a plot in each block for each possible combination of the two fertilizers. Within the blocks the plots were arranged entirely at random, and with this limitation the four blocks were replicates of one another.

In an attempt to reduce the observable colour differences to numerical terms, a single block of this experiment was used. A copy of Ridgway's *Colour Standards and Nomenclature* (17) was taken on to the field and a suitable colour scale found by a series of trials. That finally selected was Green- Yellow XVII, 27'. Direct comparison was then made with ten plants on every plot on the selected block. The plants were taken at random and to ensure a fair comparison, the pinnae examined were taken at the same position on the stem and leaf in every case. The selected position was that used for the experiments already described. It was found advisable to make the comparison by diffused light, and to secure this the scale was

shaded from direct sunshine by a hat. From the ten observations an average figure was calculated for each plot, as set out in cross columns below :

TABLE XI.

*Average Green Tone.*

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> in cwt. per acre.

K <sub>2</sub> SO <sub>4</sub> in cwt. per acre	0.	1.	2.	4.
0	6.1	6.6	7.1	8.0
1	6.2	7.2	7.1	7.8
2	6.1	6.6	7.1	7.4
4	6.0	6.5	6.7	7.2

The general result is clear without formal analysis. Increased application of ammonium sulphate leads to a deepening of the leaf colour, but the amount of potassium present is without effect. It must be remembered, however, that even though no potassium is added, a certain quantity is present in the soil, and it has in fact been observed (10) that at very low concentrations potassium has an effect on leaf colour. Such observations were obtained with water cultures and are quite impossible under field conditions.

## 8. SENESCENCE.

The change of colour associated with the senescence of the potato leaf is perfectly definite, and is not affected by such small differences as those of the above section. Although the green colour may fade before its final disappearance, a stage is reached at which a golden yellow appears, and advances a well defined frontier into the green. It is thus easy to judge by direct inspection the proportion of a leaflet that has lost its original pigment. Since the leaf is divided into a number of separate pinnae (usually seven or nine), the proportion of the leaf which has changed is also easy to observe. After yellowing the leaf undergoes a further and final colour change, when it shrivels and becomes a dark brown, and shortly after this it drops from the stem leaving only a scar to indicate its existence. The yellowing and withering of the leaves may be taken as indicators of their metabolic age, and the proportion of yellow and withered leaves on the plant as a similar index to condition. If two plots receiving different treatments, *A* and *B*, are planted on the same day, the plants upon them will not reach maturity at the same time. At any intermediate date, therefore, the *A* plants will be at different stage of their life-cycle from that of the *B* plants. In the case of individuals their relative positions could be assessed during the later phases by determining the amount of yellow leaf surface (*Y*) that had appeared. The shorter lived plant would show the greater amount of colour



change. In dealing with plots and controlled treatments the same method could be applied to a sample of plants taken from each plot at random. The further change to brown could be taken into account in two ways. In the first place browned leaves, including those which had dropped from the plant, could be considered as having fully yellowed and the further changes be ignored in calculating the 'yellow index',  $Y+B$ . The number of brown leaves ( $B$ ) could also be taken as an independent measure of age, and the two estimates be compared. Alternatively a brown leaf could be regarded as indicating a greater ageing than a yellow one, and the total age index be obtained by the expression  $Y_b = Y + kB$  where  $k$  is some constant greater than unity. Both methods were tried, since the same primary data could be used in each case. To estimate  $Y$  three categories of leaves were taken into consideration, fully yellow, more than half yellow, and less than half yellow; the values 1, 0.75, and 0.25 being assigned to these respectively. The separation of the leaves into these classes by inspection offered no difficulty, and  $Y$  was then obtained for each plant examined by adding together the class values, thus:

$$Y = \begin{array}{ccc} \text{number} & \text{number} & \text{number} \\ \text{fully} & + \text{more than} \times 0.75 & + \text{less than} \times 0.25. \\ \text{yellow} & \text{half yellow} & \text{half yellow} \end{array}$$

$B$  was obtained by counting the number of withered leaves attached to the plant, and adding the number of leaf-scars which could be detected at the base of the stem. Once withering commenced its progress over the leaf was so rapid that it was difficult to determine categories such as those applied to yellowing, and only whole numbers could be dealt with. The arbitrary value of 2 was assigned to  $k$ .

The first series of figures obtained were treated according to both methods of estimating ageing, the results of which were found not to differ in any important respect. The relations between different treatments, that is to say, were identical, whichever method of estimation was used. Counting was carried out on the 'Quantitative Experiment' upon three different occasions, July 23, August 11 and 27. The first two counts were made on block  $B$ , and the third on block  $D$ . On each occasion the treatments examined were 0, 1, 2, and 4 cwt.  $K_2SO_4$  per acre with no addition of ammonium sulphate. On July 23 ten plants taken at random were examined on each plot, but on each of the subsequent occasions this number was increased to twenty. In each case  $Y_b$  was calculated and the estimates analysed statistically. To test the significance of the differences between treatments, each occasion was taken separately, since the number of observations was not always the same. On July 23, 39 degrees of freedom were available from the forty plants examined, three of them being ascribable to treatment and the remaining thirty-six to uncontrolled, or 'random' causes. On both

the following occasions there were 79 degrees of freedom in all, with three still belonging to treatment, and 76 being random. The tests for significance gave the following results :

TABLE XII.

*Analysis of Variance of Leaf Ageing.*

	Sums of Squares.	Degrees of Freedom.	Variance.	Log <sub>e</sub> Variance.	$Z$ Found.	$Z$ For $P=0.05$ .
July 23. Treatment	156.1547	3	52.0516	3.9522	1.0984	0.47-0.55
Remainder	208.3437	36	5.7873	1.7555		
Aug. 11. Treatment	632.2461	3	210.7489	5.3504	1.1839	0.4787
Remainder	1500.5281	76	19.7438	2.9826		
Aug. 27. Treatment	10069.9898	3	3356.6632	8.1189	0.6383	0.4787
Remainder	71177.7344	76	936.5497	6.8422		

On each occasion there is a clear indication that the value of  $Y_b$  is dependent upon the quantity of potassium sulphate supplied, and hence that this substance affects the length of the life-cycle of the potato plant. In Fig. 6 are given curves illustrating the mean values of  $Y_b$ . It will be seen that on each occasion the middle range shows a reduced amount of yellowing compared with both the high and low extremes. The treatment variance was, therefore, further analysed as follows :

TABLE XIII.

	Sums of Squares.	Degrees of Freedom.	Variance.	Log <sub>e</sub> Variance.	$Z$ Found.	$Z$ For $P=0.05$ .
July 23. 0 and 4 with 1 and 2 Other Com- parisons	153.7656 2.3891	1 2	153.7656 1.1946	5.0357	1.6401	0.67-0.72
Aug. 11. 0 and 4 with 1 and 2 Other Com- parisons	603.4258 28.8203	1 2	603.4258 14.4102	6.4026	1.7100	0.6729
Aug. 27. 0 and 4 with 1 and 2 Other Com- parisons	8226.5820 1843.4078	1 2	8226.5820 921.7039	9.0856	1.1217	0.6729

By comparing these variances with the appropriate remainder variances in Table XII, values of  $Z$  were obtained as shown. On each occasion the great bulk of the total treatment variance is contributed by the comparison between the middle and extreme values of the range, and only a very small proportion is attributable to other comparisons. As might be expected, therefore, the variance of the selected comparison is shown to be undoubtedly significant, while those of other comparisons are not.

An interpretation of the curves of Fig. 6 seems, therefore, clear. They indicate a case of 'physiological balance', the importance of which has

already been shown in numerous instances. At the medium concentrations potassium is co-ordinated with other substances present in a manner favourable to a prolonged life-cycle, and yellowing and the senescence it indicates are, therefore, postponed. At the higher concentration this balance is again lost, and yellowing is as fast or faster than with a deficiency. A second possible explanation can be disposed of. If the total number of leaves on the plant was also smaller on the 1 and 2 cwt. plots than on the others the form of the curves might simply be due to a uniform yellowing proportional to the total number of leaves produced. In the analysis of leaf number on p. 178, it is shown, however, that there was a significant reduction of leaf number at the top and not at the bottom of the concentration range. These results were obtained at the same time, and using the same plants as the yellowing data. The same result can be shown by calculating  $Y_b$  as a percentage of the total leaf number. If the explanation is true, the curve should then become a horizontal straight line, independent of concentration. Actually (see Fig. 6, broken curve) only a relatively unimportant deviation from the former curve is introduced. It thus makes little difference whether  $Y_b$  is expressed as an absolute amount or as a percentage of the total number of leaves.

A further aspect of senescence which, on account of its visible results, may be called 'coppering', is brought into sharp relief by these methods. About the beginning of August some of the plants began to develop minute brown spots on their leaves. These were at first extremely small, and scattered irregularly about the laminae, though somewhat more numerous towards the edges. They involved all the internal layers, passing right through the leaf and appearing at a corresponding point on the other surface. With time these spots extended and coalesced into brown, withered patches of irregular form and size, the tissues of which were obviously dead. It was noticeable as they began to enlarge that they were formed, and for a long time restricted, within the islands lying between the veins. Not until a comparatively late stage did they invade bundles large enough to be visible. This method of development is in sharp contrast to the withering that follows the change to yellow. In the latter case the leaf turns brown in much larger patches which work inwards from the border, instead of originating all over the lamina. Coppering, moreover, is not necessarily or even typically associated with yellowing. Whereas the latter starts at the lower leaves and only slowly works its way upwards, coppering appears upon the younger leaves at the top of the haulms, and usually at a stage when they are still quite freshly green. Its occurrence in lower leaves is very much rarer.

A general inspection of the potato plots suggested a close connexion between coppering and a low potassium supply. On August 27, when the change was much in evidence, a count was made simultaneously with those

for yellowing, of the number of affected leaves on each plant. There were thus obtained figures for a sample of twenty plants on each of four plots receiving 0, 1, 2, and 4 cwt. potassium sulphate per acre respectively.

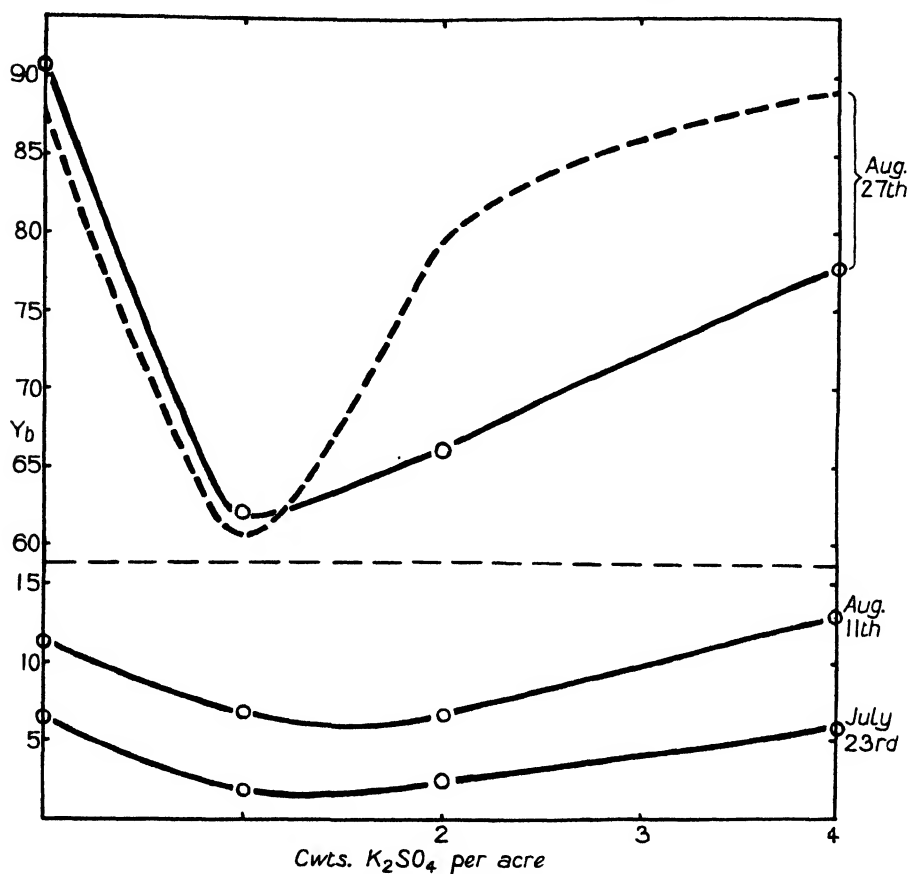


FIG. 6. Age index,  $Y_b$  (see p. 190), for three different periods and four different treatments. The curved broken line shows the index expressed as a percentage of the total number of leaves. The scale of ordinates is interrupted between 15 and 60.

Analysis of the variation observed gave the following results:

TABLE XIV.  
*Analysis of Variance of Coppering.*

	Sums of Squares.	Degrees of Freedom.	Variance.	Log <sub>e</sub> Variance.	Found. $Z$	For $P = 0.05$ .
Treatment	1064.8	3	354.93	5.8718	1.3078	0.4787
Remainder	1972.0	76	25.95	3.2563		
Totals	3036.8	79				

A significant reduction in the number of affected leaves is thus associated with an increase in the supply of potassium. That the reduction is not dependent to any great extent upon the simultaneous reduction of the total leaf number is shown by its magnitude. The number of leaves on the untreated plot did not exceed those on the 4 cwt. plot by as much as 20 per cent., whereas the number affected by coppering was ten times as great. Another count carried out on August 11 gave similar results with smaller numbers. On the other hand, no such reduction was found when ammonium sulphate was investigated, and there seems every reason to suppose that coppering, or local death of the leaf tissues, is due to an insufficient supply of potassium.

## 9. DISCUSSION.

The foregoing results, positive and negative, present a picture of some complexity, and one is forced to conclude that the part of potassium in the leaf is not a simple action, but rather a manifold activity. It is now well established that a deficiency of potassium<sup>1</sup> reduces the amount of reserve carbohydrate precipitated by the plant, and the main object of this discussion is to examine the mechanism which brings the reduction about. In the early part of the paper it was shown that adequate potassium manuring causes an increase of efficiency, rather than a multiplication of the photosynthetic machinery. Leaf area is, if anything, reduced by additions of potassium which simultaneously cause larger amounts of starch to be formed in the leaves themselves and in the reserve organs. Laying aside mere increase of leaf surface, attention may, therefore, be concentrated upon the question of efficiency. This may vary in any or all of the stages lying between the uptake of carbon dioxide and the final appearance of starch<sup>2</sup>. For the purpose of the present analysis the following stages may be recognized :

1. The diffusion stage in which carbon dioxide travels to the chloroplast surface. Carbon dioxide concentration is here pre-eminently the controlling variable.
2. The photochemical stage upon which light exerts a controlling influence, and
3. The 'dark reaction' stage, which is subject to the influence of heat.

As a result of these reactions hexose sugars are produced, which may then undergo further modifications, the principal of which is condensation to starch. This may be called the fourth or condensation stage of the series. The first step in the removal of starch from the leaf is assumed to be its reconversion to a hexose sugar, and translocation must not therefore be separated from the question of starch formation.

<sup>1</sup> It is not formally shown by the experimental results that the observed effects are due to potassium and not to the associated sulphate. The evidence of yield experiments is strongly in favour of the kation.

None of these stages is *a priori* ruled out of the problem. In the first, potassium might materially increase the supply of carbon dioxide to the chloroplast by the formation of a bicarbonate buffer in the cytoplasm. It is improbable that such buffers occur, however, since it has been shown that the pH of plant cells is rarely alkaline (Atkins, 1 and 2), and the active intervention of potassium at this stage cannot be substantiated. Evidence of its effect in the photochemical and dark stages is, however, forthcoming from the work of Briggs (5), who found that bean seedlings deprived of potassium showed reduced rates of oxygen production, both when light and when temperature were limiting. Accelerations in these stages, to whatever cause they were due, would lead to an increased production and concentration of hexose, which in its turn would promote a greater formation of polysaccharide. The observation of increased quantities of starch in the leaf does not, therefore, necessarily indicate that the condensation stage itself has been made more efficient, since a faster rate of any step in the series will lead to a quickening of them all. That the efficiency of this stage is directly effected becomes probable, however, from a consideration of starch removal. Both from the work of Maskell (14) and from the present paper, there is reason to suppose that this is also accelerated. The velocities of the hexose-starch and starch-hexose reactions are dependent upon a number of factors, among which may be named temperature, concentration of hexose, and concentration of enzyme. Starch, being only slightly soluble, occurs as a saturated solution in the presence of its solid phase, and its effective concentration is thus kept constant. Fluctuations in the total amount of starch in the leaves are not, therefore, reflected in the velocity of the starch-sugar reaction. Light also is without direct effect, as this stage of the process is able to continue in the dark if sugars are artificially supplied. As temperature may further be ruled out, any effect of potassium upon the rates of the reactions may be expected to take place through the concentrations of hexose or enzyme. If the opposing reactions are supposed to constitute a single reversible reaction, a reduced hexose concentration during a period of darkness might be expected to lead to a faster disappearance of starch. It has been shown by Mason and Maskell (15) that translocation always occurs from high to low concentrations of sugar, and that the rate of translocation is proportional to the fall in concentration. Starch removal might, therefore, be accelerated by a reduction of hexose concentration in the leaves, accompanied by a corresponding reduction in the stems and final storage organs, if any. In the Rothamsted field experiments of 1900 and 1902 (12) it was found that the addition of potassium sulphate led to a larger formation of hexose sugars in the roots of man-golds. Beal and Muncie (4) found that application of the same substance raised the concentration of sugars in sap pressed from the leaves and stems of carnations. The acceleration observed in the removal of starch is,

therefore, not likely to depend on a reduced hexose concentration, and we are driven to explain the additional hydrolysis as due to an increased activity of the appropriate enzyme series, which is diastase.

If, on the other hand, one accepts the evidence which has been brought forward to prove starch condensation and hydrolysis two independent reactions, hexose concentration can have no direct effect upon the rate of starch decomposition, and again, the action of potassium can only be through the hydrolytic enzyme. This explanation is made even more probable by the demonstration that diastase *in vitro*, when completely separated from neutral salts becomes inactive, and that its activity is restored by addition of salts of potassium and other metals (Vulquin and Lisbonne, 19). The results of Kendall and Sherman (13) and others make it highly probable that diastase accelerates the condensing reaction as well as the hydrolysis, and the presence of potassium will therefore influence them both.

There is thus reason to believe that potassium directly affects three of the four stages in starch formation, and it would be interesting to know whether this is done by similar or widely different means. In the last stage, it has been suggested, there is an increased effectiveness of catalytic surface, but the manner in which this is secured is not clear. The alternative possibilities appear to be four. There may be an actual increase of the enzyme surface owing to (1) a greater dispersion of its material, or (2) the formation of entirely new enzyme; or there may be an increased efficiency of a unit area of the surface already existing, (3) by an increase of its adsorbing or combining power, or (4) by a change in the degree of dissociation.

In his examination of the photochemical and dark stages of assimilation, Briggs came to the conclusion that the effect of potassium and other metals was to increase the active chloroplast surface, and a certain amount of unity can thus be recognized in the potassium effects in the various stages of carbohydrate metabolism, since they are always concerned with an increase of catalytic powers.

None of the facts elucidated in the foregoing paper or otherwise known to the author is in any way at variance with this conclusion. It is not supposed, however, that this represents the sum of the activities of potassium ions in the plant, and the effect upon the rate of yellowing is a case in point. The ageing of leaves has been found to be correlated with the disappearance of such diverse substances as water (8), nitrogen (6), carbohydrates, chlorophyll, and many mineral elements of which potassium (7) is one. Most of these pass into the axes of the plant, others such as chlorophyll are degraded *in situ*. In most instances the reason for migration or decomposition is quite unknown, and cause cannot yet be distinguished from effect. Lack of carbohydrate is presumably a result of leaf degeneration, not a primary cause of it. With the ash elements the case is not so clear, and it is there-

fore interesting to find, as shown above, that an increased supply of potassium will delay the breakdown of the mechanism. Here, it seems, is a factor which can definitely be said to be controlling, not controlled.

To describe the way in which this is done is another matter. In a process governed by so many factors, known and unknown, the attempt, without further knowledge, can only lead to disappointment.

#### 10. SUMMARY.

1. In order to investigate the physiological importance of potassium, field experiments were carried out upon certain attributes and functions of potato leaves. Number, area, weight, water content, and rates of starch formation, translocation, and senescence were examined. The primary data were subjected to statistical analysis, and the following conclusions arrived at.

2. The number of leaves formed on an average per plant was found to be significantly reduced by the application of potassium sulphate, or 'potash manure salts', a low-grade fertilizer. Potassium chloride could not be shown to have any effect.

3. Area of a selected leaflet. The area of the penultimate pinnae of the fourth leaf from the stem apex was not affected in adult plants by the addition of potassium sulphate, but addition of 'potash manure salts' or potassium chloride caused an increase of surface. This is ascribed to the action of the chloride ion present in both the latter fertilizers. Taken in conjunction with the reduced leaf number, the lack of effect of the sulphate suggests that potassium itself tends to decrease rather than increase the total leaf area of the plant. There is, however, some evidence of an increase in the earliest stages of growth.

4. Leaf water content, expressed as water weight / dry weight, showed no significant response to potassium manuring. The presence of chlorides, however, again caused an increase. It is shown that a very high correlation exists between leaf area and the water weight / dry weight ratio, and the increase of leaf area due to chlorides is probably brought about by an increase of water content.

5. Dry weight of the selected leaflet was found to be unaffected by the addition of potassium compounds.

6. Starch formation per unit leaf area showed a significant increase in response to potassium, particularly when in the form of sulphate. There was little or no response to the presence of chlorine.

7. Translocation could not definitely be shown to be affected by the same treatment, but reasons are given which make it probable that an acceleration in its rate is brought about

8. Senescence, as indicated by the yellowing of the leaves, was delayed



by the addition of one or two cwt. of potassium sulphate per acre. Four cwt. per acre did not have a similar effect. In all these concentrations there was no detectable effect on the colour of healthy green leaves. 'Coppering', a characteristic spotting of young foliage, was shown to be clearly related to a deficiency of potassium.

9. These points are discussed, and it is shown that one important effect of potassium in leaves is an increase of catalytic activity, leading to greater efficiency in three of the four stages of starch formation. It is further suggested that loss of potassium is a causal factor in leaf ageing.

The author wishes to express his great indebtedness to Dr. R. A. Fisher for his invaluable advice on the statistical handling of the data, and to the many willing helpers who made their collection possible.

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# Increased Scion Vigour Induced by Certain Foreign Root-stocks.

BY

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With Plate XXXVIII and one Figure in the Text.

IN the course of grafting experiments made by the author at the Rothamsted Experimental Station, and designed to throw light on the causes of the limitation of certain parasites and symbiotic bacteria (2, 3) to definite host plants, combinations were observed in which the scion made better growth on a foreign root-stock than on its own. These observations seem sufficiently interesting to be collected together in spite of the fact that they were made incidentally to other work, and so are not as complete as could be desired for the present purpose.

## I. WOODY NIGHTSHADE ON POTATO.

In the summer of 1922 a number of equal sized cuttings taken from a single plant of woody nightshade (*Solanum Dulcamara*) were rooted in moist soil in 10-in. pots; of these rooted cuttings five were selected as being as uniform in size, vigour, &c., as possible. Two of these were set aside as controls; the other three were cut off just above soil-level and grafted on single-stem potato (*S. tuberosum*) plants which had been obtained by tearing from the parent tuber a single rooted shoot as soon as leaves appeared above soil, and planting it in a 10-in. pot.

The grafts took readily and, in spite of starting off shorter because of the bits of stem removed in the operation of grafting, they soon outgrew the two controls. In Pl. XXXVIII, Fig. 5, are shown the two control plants and one grafted one. The leaves of the grafted plants were three or four times the area of the control ones and of a much more luxuriant appearance. The axillary bud of almost every leaf on the grafted plants developed into a strong shoot, whereas the ungrafted plants were hardly branched at all. The girth of the grafted plants soon exceeded that of the control ones.

No marked differences in date of flowering or fruiting was noticed. The condition of the mature plants is shown at the top of Plate XXXVIII;

Fig. 1 is of two control plants, and Figs. 2, 3, 4 each show a single grafted plant. The two control plants were taken together. The marked increase in size of top due to grafting on potato is well seen in the illustrations.

The grafted plants were cut off just above the graft, and the controls at a corresponding height above soil-level. The weights of tops were:

Grafted plants	11 grm.	19 grm.	12 grm.	Average = 14 grm.
Control plants	5 "	7 "		" = 6 "

Hence the tops of the grafted plants were on the average more than twice the weight of the ungrafted ones. The grafted plants also produced potato tubers below ground, making the increased vigour of the top the more remarkable.

*Potato on woody nightshade.* At least a dozen grafts were made of potato on woody nightshade, in which apparently satisfactory organic union took place. Even when dry sand was held round the stem to allow of natural tuber formation the potato scion remained very stunted.

## II. *VICIA FABA* (BROAD BEAN) ON *V. NARBONENSIS* GRAFTS.

In an attempt made with Dr. J. Davidson to study the nature of the resistance of *Vicia narbonensis* and the extreme susceptibility of *V. faba* to aphid attack, a number of grafts between these two plants were made in order to ascertain the effect on the aphides of grafting a foreign root-stock to their host plant. The main results of this work have not yet been published. The following observations were made incidentally during the course of the experiments.

### 1924 Experiment.

Seeds were sown in soil in 10-in. pots, three seeds to a pot. At the time of grafting the seedlings were thinned to one per pot, the thinning being done so as to give as uniform a set of seedlings as possible. The *V. narbonensis* seed was sown two or three days before the *V. faba* seed to counteract somewhat the difference in circumference of the stems. Cleft grafts were made as described elsewhere (2, 3) when the first foliage leaf was opening.

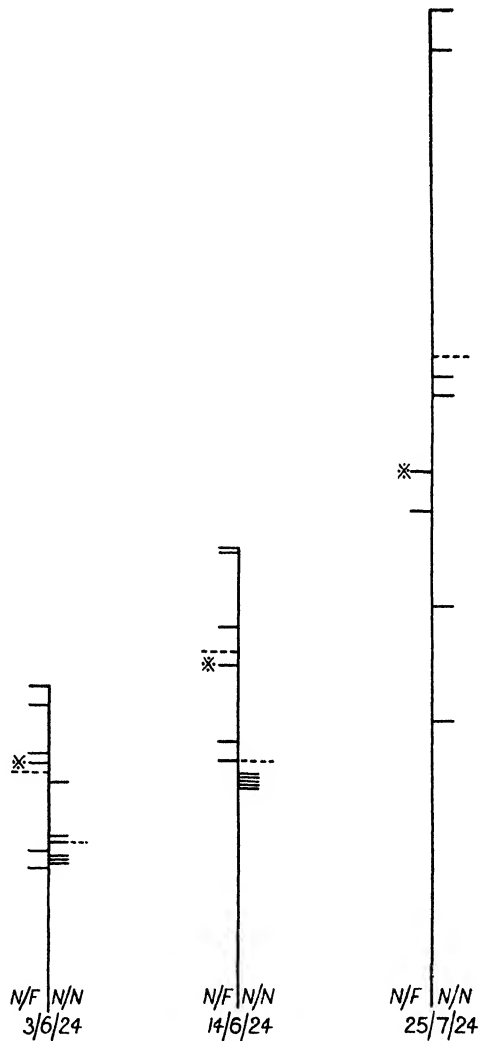
The heights of the plants are shown in the text-fig. The grafts were made on 23/5/24. By 3/6/24 the plants grafted on *V. faba* had outstripped the ones grafted on *V. narbonensis* root-stock, the average height of the one (12.6 cm.) being 1.4 times that of the other (8.8). On 14/6/24 the average heights were 18.8 cm. and 13 cm. respectively, the plants on *V. faba* again being 1.4 times as high as those on their own roots.

Unfortunately, before the next records were taken, on 25/3/24, four plants had died, leaving only two *V. narbonensis* on *V. faba* grafts alive

and healthy, but all six self-grafted *V. narbonensis* plants were healthy. Their average height of 34 cms. was well above that of the one marked \*, i. e. 28 cm., which on the two previous occasions was almost exactly an average specimen of the *V. narbonensis* or broad bean grafts. This supports the visual impression that a week or so previous to the day when the final record was taken, before any of the *V. narbonensis* on *V. faba* plants had died, they had been overtaken by the self-grafted *V. narbonensis* plants.

**Flowering.** On 16/6/24 only one self-grafted *V. narbonensis* plant showed any signs of flowering; in it only the purple tips of flowers were showing. Of the *V. narbonensis* on *V. faba* plants, however, two had one flower each nearly open, two others had one each fully open, and the other two had two flowers each fully open.

**Foliage.** The foliage of the *V. narbonensis* on *V. faba* plants was of a distinctly yellower colour and of a 'softer' appearance than the self-grafted *V. narbonensis* plants. This yellowing in the bottom leaves gave place to actual browning and final withering, even in plants which were less than half-grown, whereas the self-grafted *V. narbonensis* ones never showed the yellowing, and their bottom leaves only became brown and dry some weeks after the plants had reached their full height.



TEXT-FIG. Heights of *Vicia narbonensis* on *V. faba* (N/F) and self-grafted *V. narbonensis* (N/N). The grafts were made on 23/5/24. Averages represented by dotted lines.

### 1925 Experiment.

When a similar experiment was carried out in 1925 no plant measurements were taken until the end of the experiments because of the risk of

damaging or disturbing the aphides; inspection of the plants, however, was sufficient to confirm the deductions drawn from the 1924 experiment, viz. the *V. narbonensis* on *V. faba*, plants at first grew faster than the self-grafted *V. narbonensis* ones, but later they lost their lead. The appearance of the 1925 plants almost suggested that, apart from disease which tended to attack the *V. narbonensis* on *V. faba* plants at the graft unions and at soil-level, they would have been slightly shorter when mature than the self-grafted ones. The *V. narbonensis* on *V. faba* plants flowered earlier than the self-grafted *V. narbonensis* plants, and again were the same curious colour as the 1924 plants.

*V. faba* on *V. narbonensis* grafts. *V. faba* took readily when grafted on *V. narbonensis*, and remained healthy-looking, but in no single graft did one approach in size a self-grafted one.

### III. LUPIN ON BROAD BEAN GRAFTS.

In 1924 Mr. H. G. Thornton and the writer commenced a series of experiments to determine whether the specific relationship existing between a leguminous plant and its nodule-forming organism is influenced by grafting a foreign top on the plant. It was noticed that lupins grafted on broad bean root-stocks grew better than when self-grafted, and even than ungrafted lupin plants.

In Pl. XXXVIII, Figs. 8 and 9, are shown two pairs of plants, the left-hand one in each being an ungrafted lupin plant, and the right-hand one a lupin grafted on broad bean. The difference due to grafting on broad bean hardly needs comment, increase in stem girth, amount of branching, and size of leaves being well seen.

In 1927 a more extensive series of experiments was carried out. The plants were grown in sand and watered with culture solution. Each pot contained one ungrafted broad bean, one self-grafted broad bean (1 only had died), 2 broad beans on lupin plants (only 7 survived to the end of the experiment), 2 lupins on broad bean plants (15 survived), one self-grafted lupin plant (6 only survived), and one ungrafted lupin plant (all of these survived).

The following notes were taken when the plants were washed out to search for nodules on the roots (see Pl. XXXVIII, Figs 6 and 7, in each of which from left to right are two lupins grafted on broad bean plants, one self-grafted lupin, one ungrafted lupin). As none of the lupin roots had nodules on them, for fairness in comparison and simplicity of presentation only those broad bean roots which remained uninfected are considered, although the inclusion of the infected plants would make little, if any, difference to the general conclusions.

Every self-grafted lupin was smaller than its ungrafted fellow. Twelve

lupins on broad bean plants (including one with slightly-diseased roots) were each larger than the corresponding ungrafted lupins; one more was larger than the corresponding self-grafted lupin, but smaller than the ungrafted one. Two, including one with slightly diseased roots, were smaller than the corresponding ungrafted lupin, but larger than all the self-grafted lupins. (The self-grafted lupins actually corresponding to them had died.)

*Broad Bean on Lupin Grafts.* Broad beans, when grafted on lupins, remained stunted, although, as far as could be judged from a naked-eye examination, satisfactory organic union had taken place.

#### DISCUSSION.

Darwin, in 'Animals and Plants under Domestication' (vol. i, p. 147), states: 'According to Mrs. Abbey, grafts or buds generally take on a distinct variety or even species . . . with greater facility than on stocks raised from seeds of the variety which is grafted: and he believes this cannot be altogether explained by the stocks in question being better adapted to the soil and climate of the place. It should, however, be added that varieties grafted or budded on very distinct kinds, though they may take more readily and grow at first more vigorously than when grafted on closely-allied stocks, afterwards often become unhealthy.' Darwin was referring to woody grafts, but it is interesting to note how closely the results of grafting *V. narbonensis* on *V. faba* are in agreement. There was little, if any, evidence in the lupin on broad bean grafts to suggest a loss of health in the mature plant. Certainly the woody-nightshade scions grafted on potato retained their enhanced vigour to the end of the season. Any more detailed comparisons of the foregoing results with those of woody grafts, as summarized by Hatton (3), though tempting, would hardly serve a useful purpose at this stage.

#### SUMMARY.

Three examples are described of a scion being more vigorous when grafted on a foreign root-stock than on its own.

Woody nightshade (*Solanum Dulcamara*) attained more than twice its normal weight when grafted on potato (*S. tuberosum*), and assumed a more branching habit. *Vicia narbonensis* at first grew abnormally tall when grafted on *V. faba*, and then was overtaken by the self-grafted plants, becoming unhealthy before reaching maturity; flowering was earlier.

Lupin when grafted on broad bean was of greater girth and height than when on its own roots.

In the three reciprocal grafts the root-stock had a dwarfing effect.

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## EXPLANATION OF PLATE XXXVIII.

Illustrating Dr. W. A. Roach's paper on Increased Scion Vigour Induced by  
Certain Foreign Root-stocks.

Fig. 1. Two ungrafted woody nightshade (*Solanum Dulcamara*) plants.

Figs. 2, 3, 4. Single woody nightshade grafted on potato plants.

Fig. 5. Early stage of the above plants. Left to right: two ungrafted woody nightshade plants, one woody nightshade grafted on potato plant.

Figs. 6 and 7. Left to right: two lupins grafted on broad beans, one self-grafted lupin, one ungrafted lupin.

Figs. 8 and 9. Left: Ungrafted lupin. Right: Lupin grafted on broad bean.

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# A Note on the Dichotomous Branching of the Main Stem of the Tomato (*Lycopersicum esculentum*).

BY

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With three Figures in the Text.

FOR the purposes of the investigations on Virus Diseases carried out in this laboratory it is necessary to have a continuous supply of tomato plants. These are grown from seed and the seedlings are planted out in pots as required. It has been noticed that there is a tendency for the first foliage leaves to have an abnormal structure. One type of variation is not uncommon. The distal portion of the leaf is frequently forked so that the terminal pinnac are 'double' and the rachis is usually split so that there are two equal terminal pinnae. When this is so the two sides of the leaves are quite symmetrical. Among the plants under observation in the glass-house, however, appeared the present one in which the 'dichotomous' habit was much more evident.

The occasional appearance of forked leaves suggests that there is a tendency in the case of the tomato to have abnormal cell-divisions of the apical meristem which give rise to apparent dichotomy, and the occurrence of this plant indicates that this abnormality is not impossible of occurrence even in the stem apex.

The tomato plant has normally a three-fifths leaf-divergence and the branching is monopodial. In the plant under observation the first few leaves and the lower part of the stem presented a perfectly normal appearance. Almost immediately above the fourth leaf, however, the stem divided equally and gave the appearance of a dichotomously branching plant. There was no further dichotomous branching and the normal monopodial structure was continued. An examination of the two limbs of the plant showed that they were identical as regards both the number of the lateral members and the places of their insertion on the stem. It will be seen from Fig. 1 that each leaf on one branch has its fellow on the other.

Cases of simple dichotomy in Dicotyledons have from time to time been recorded. Worsdell (1) cites examples viz. the stems of the Jerusalem



FIG. 1. Tomato plant showing the bifurcating stem.

Artichoke (*Helianthus tuberosus*), of *Maesa ramentacea*, of Wall-flower (*Cheiranthus cheiri*) and the Stonecrop (*Sedum reflexum*).

In order to decide definitely that the present example was not merely an apparently equal development of the main stem and of an axillary shoot a study of the anatomy was made. Fig. 2 indicates the appearance of the stem at the place of forking. The leaf and its accompanying bud are clearly visible just below the region of division. This practically disposes of the possibility of one of the stems being axillary in origin. On the opposite side of each of the stems behind the rather swollen node are leaves each with an axillary bud, equal in size and corresponding in position. These represent the fifth leaf of the plant.

Further evidence that the stem was not axillary in origin was furnished by the examination of the vascular anatomy. In the tomato stem there are main groups of common bundles with connecting vascular tissue, forming a continuous stellar ring. When an axillary shoot grows out the vascular connexions are made entirely from the common bundle with which is connected the leaf in the axil of which it arises. It is thus a simple matter to recognize the out-growth of a lateral shoot from the examination of the vascular tissues of the stem.

In the present specimen there was a bud in the axil of the fourth leaf so that a few sections in this region sufficed to give examples of this type of vascular connexion. The vascular arrangement in the region of bifurcation is very different. The sections in Fig. 3 illustrate the salient features of the two types. The first section (3*a*) was made just below the fourth leaf, the second (3*b*) in the region of the fourth leaf, the third (3*c*) between this leaf and the fork, and the fourth (3*d*) at the lower end of the fork. Just above the fourth leaf the stem becomes rather flattened and increases in width in a direction at right angles to the length of the leaf. Thereafter a split appears in the vascular tissue, possibly associated with the difference in constitution of xylem tissue at this place

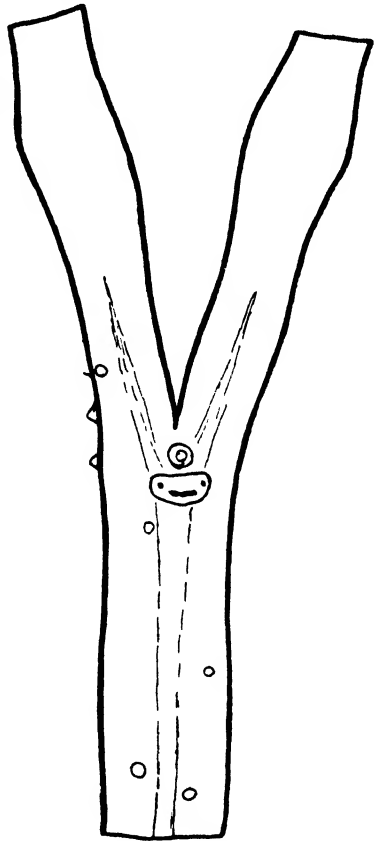


FIG. 2. Diagram of stem in region of forking.

and the initiation of the vascular tissues of two stems is evident. The point of interest is that in this case the two systems arise by the splitting of the stele of the stem. One is not formed, as in the case of an axillary shoot by the outgrowth of tissue from one of the common bundles of the main stem. Actually the division of two of the main vascular groups are to be seen in the sections. Each of the two limbs of the fork is therefore a true 'main stem'.

The evidence indicates, that in the present instance, the forking was due to a dichotomous division of the main stem and not to the over-growth of a lateral shoot with simulated dichotomy. The fact that the two stems are identical may be fortuitous or it may indicate that, the meristem having divided equally at an early stage, the resultant halves developed in exactly the same way. The latter seems the more probable explanation. The

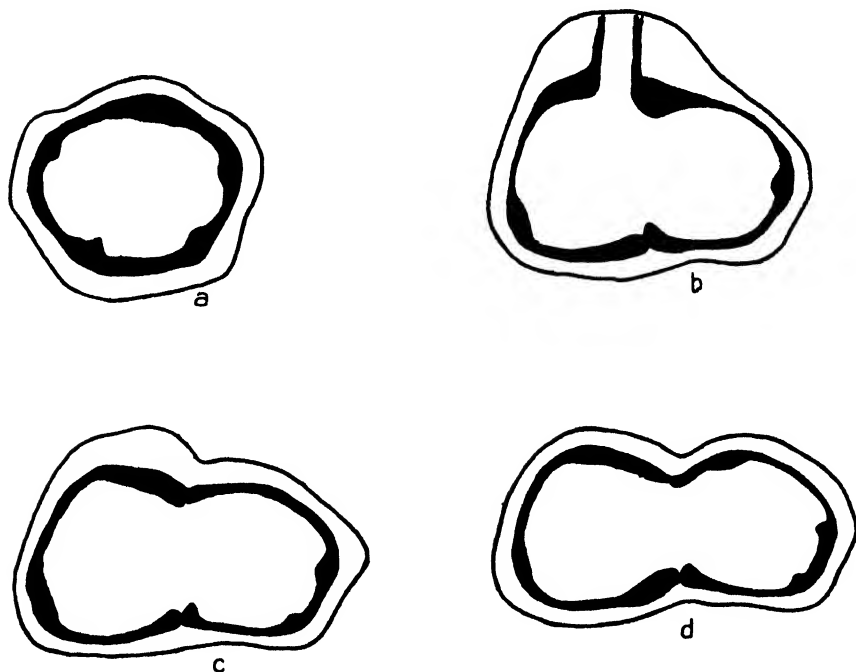


FIG. 3. Diagram of transverse section of stem at different levels. (a) 'Normal' main stem; (b) in region of fourth leaf; (c) between fourth leaf and fork; (d) at base of fork.

development of each of the parts seems to have followed the same course as would have been followed by the main stem had it continued normal growth under similar circumstances.

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# The Early Development of the Root Nodule of Lucerne (*Medicago sativa*, L.).

BY

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With Plates XXI and XXII and one Figure in the Text.

## A. HISTORICAL.

MANY authors have described the histology of nodules on leguminous plants and their development. Their descriptions, however, disagree materially, while there are still important points which require elucidation. There is considerable variation in the course of nodule formation in different host legumes, which largely accounts for the disagreement. Spratt (16) studied the anatomy of nodules on some 23 genera of legumes, and divided them into four groups according to the distribution of the vascular supply, meristem, 'bacteroidal tissue', &c. Milovidov (10, 11) found important differences in the method by which bacterial infection of the host tissue is effected. He describes three types of infection. (a) In the majority of the leguminosae infection of the cells is produced by means of the characteristic 'infection threads' containing the bacteria, which intrude into the young cells produced in the meristem of the nodule. (b) In the type characteristic of *Serradella*, intercellular zoogloea plays the principal part in the infection. (c) In *Lupinus*, active division of infected cells is the principal means by which the 'bacteroidal tissue' is increased. The last type of infection is also characteristic of *Phaseolus* (8). The nodules on lucerne, here considered, belong to the first type, most, if not all the cells of the infected region receiving their bacteria by separate intrusions of the infection thread.

The general course of infection and of nodule formation in this type is well known, perhaps the best description of it being that given by Pierce (13) in the case of Burr clover. There are still important details, however, which are not understood. The bacteria normally enter the plant by penetrating the root-hairs near their distal extremity, although there is evidence that they may, more rarely, enter the other epidermal cells (3).

The plant secretes from the roots a substance which assists this infection (17), but the small percentage of root-hairs infected even in the presence of large numbers of bacteria indicates that the plant tissues can resist excessive infection (18). It is not known how the bacteria penetrate the wall of the root-hair. No cellulose-splitting enzyme can be detected in cultures of the organism, but infected root-hairs usually show a characteristic curling of the tip, which according to Hiltner (7) can be induced by means of a bacteria-free filtrate of a culture. The point of infection is generally in this curled region.

The bacteria within the root-hair form one or more 'infection threads' which grow down the hair and penetrate the cortical cells of the root. Observers have disagreed as to the structure of this infection thread. Earlier workers thought that the threads were fungal hyphae and were enclosed in a cellulose wall. Prazmowski's observations in 1890 (14), showed that the thread contained bacterial rods, and since then there have been two opinions as to their nature. Pierce (13) and Fred (6) thought that they consisted of strands of bacterial zoogloea, while (Dawson) (5), Burill and Hanson (3), and Dangeard (4) regarded the thread as a tube possessing a definite sheath containing the bacteria. The means by which the bacteria in the infection threads penetrate the cortical cell walls is not known. They often show funnel-shaped expansions at the point of contact with the wall, but opinions differ as to the significance of these.

Penetration of the cortical cells is accompanied by their rapid division, producing a mass of young cells through which the infection threads ramify. It is not clear how this cell division is induced. Some observers state that infection is limited to cells outside the endodermis, while others claim that the pericycle cells are infected. Probably this differs according to the species of host plant.

Some of the bacteria escape from the infection thread and come to lie scattered in the cytoplasm. The means by which they escape is difficult to follow, because at this stage the bacteria are very small and closely resemble the mitochondria of the cells. After their escape, the bacteria multiply rapidly and increase in size, usually becoming irregular and branched, the so-called 'bacteroid stage'. Abundance of this bacteroid stage seems to be correlated with active nitrogen fixation within the nodule.

Observers differ as to whether penetration of the cells by the infection thread immediately arrests cell division, but as soon as the bacteria become numerous in the cytoplasm the host-cell ceases to divide, but increases in size with a corresponding hypertrophy of the nucleus. The effect of the bacteria upon the infected cells has been much discussed (McCoy (8)); it is now known that this differs according to the physiology of the host plant (2) and (19), and the age of the bacteroidal tissue (9) and (19).

The following observations on the early development of the nodule in



lucerne (*Medicago sativa, L.*) were made in the hope of elucidating some of the points of uncertainty above mentioned.

### B. TECHNIQUE.

Lucerne seedlings were grown in wide test-tubes containing an agar medium made up by adding the following ingredients to 1,000 c.c. of distilled water :

$K_2HPO_4$	.	.	.	0.5	gram.
$MgSO_4 \cdot 7 H_2O$	.	.	.	0.2	gram.
NaCl	.	.	.	0.1	gram.
$Ca_3(PO_4)_2$	.	.	.	2.0	gram.
$FePO_4$	.	.	.	1.0	gram.
$FeCl_3$	.	.	.	0.01	gram.
Agar	.	.	.	10.0	gram.

The tubes of medium were sterilized in the autoclave, and each was sown with two lucerne seeds the coats of which had been sterilized by immersion in absolute alcohol followed by 0.2 per cent.  $HgCl_2$ , washed off with several changes of sterile water. As soon as germination took place the tubes were inoculated with a week-old culture of the lucerne nodule organism. The strain used was one of known nitrogen fixing efficiency. As soon as the first true leaves were well developed nodules began to appear, and, from this time onward, nodules of various ages were fixed in Bouin's fixative (1). Sections  $5 \mu$  thick were made, and these were stained with iron haematoxylin and orange G. Some sections were also stained with carbol fuchsin, which emphasizes the bacteria but does not bring out other structures so clearly.

### C. DEVELOPMENT OF THE YOUNG NODULE.

Infection was seen to have taken place through root hairs wherever sufficiently young nodules were examined (Pl. XXI, Fig. 1). Two or more infected root-hairs sometimes contributed infection threads to a single nodule. Curling of the root-hair tip was seen in all cases. The infection thread often branches within the root-hair, several strands passing down it. The threads penetrate the cortical cells as far as the inner layers, but do not enter the endodermis. Along their course the cells become more densely protoplasmic, their nuclei swell, and active cell division commences (Pl. XXI, Fig. 2). This division extends to a distance of two or three cells from the lines of infected cells, and is therefore induced by a diffusible substance. It occurs not only in the cortex but also to a small extent in the endodermis and pericycle.

The infection threads penetrate the young cortical cells produced by

division. The growing tip and youngest portions of the thread consist of a slime-like matrix filled with short rod-shaped bacteria about  $0.75 \times 0.5 \mu$  in size. The infection thread has no definite sheath at this stage, its edges being somewhat irregular (Pl. XXI, Fig. 2), and there is a tendency for it to swell into zoogloea masses. When the growing tip reaches a cell wall it often swells out to form a small mass of zoogloea applied to the wall, which is penetrated at a spot somewhere near the centre of the area of contact. When the bacteria pass into the new cell they may produce a similar zoogloea mass on the other side of the wall (Pl. XXI, Fig. 5), from which the infection thread continues to grow. It is probably the subsequent shrinking of these zoogloea masses on either side of the wall that produces funnel-shaped expansions characteristic of older portions of the thread at the points where they cross cell walls (Pl. XXI, Figs. 5, 7). The growing point of the infection thread does not always swell into a mass of zoogloea at the point of contact with the wall. It may pass through the wall without changing its diameter (Pl. XXI, Fig. 4).

The infection thread, though initially naked, soon becomes enclosed in a sheath. This sheath is at first very thin, but becomes thick as the thread ages, as can be seen if the course of a thread is followed backwards from its growing point (Pl. XXI, Figs. 5 and 6). In fixed material there is usually a clear space separating the sheath from the contained strand of bacterial zoogloea (Pl. XXI, Fig. 5). This is perhaps the result of shrinkage. Older portions of the thread are sometimes almost devoid of bacteria, suggesting that the latter are able to pass down the course of the strand. This observation was also made by Dangeard (4). The sheath is continuous with and similar in staining reactions to the cell walls of the host plant, and may thus be produced by the host as a defence mechanism against the bacteria, as suggested by Moeller (12) and by Schneider (15).

The infection thread tends to grow up to and frequently applies itself against the nucleus of the host-cell, as though attracted thereto. Division of the host-cells is not immediately arrested by the entry of the bacteria (Pl. XXI, Fig. 3), since cells in a state of division can frequently be seen to contain infection threads. Such cells contain young naked portions of the thread; cell division ceases by the time the infection thread sheath is formed.

#### D. RELEASE OF THE BACTERIA FROM THE INFECTION THREAD.

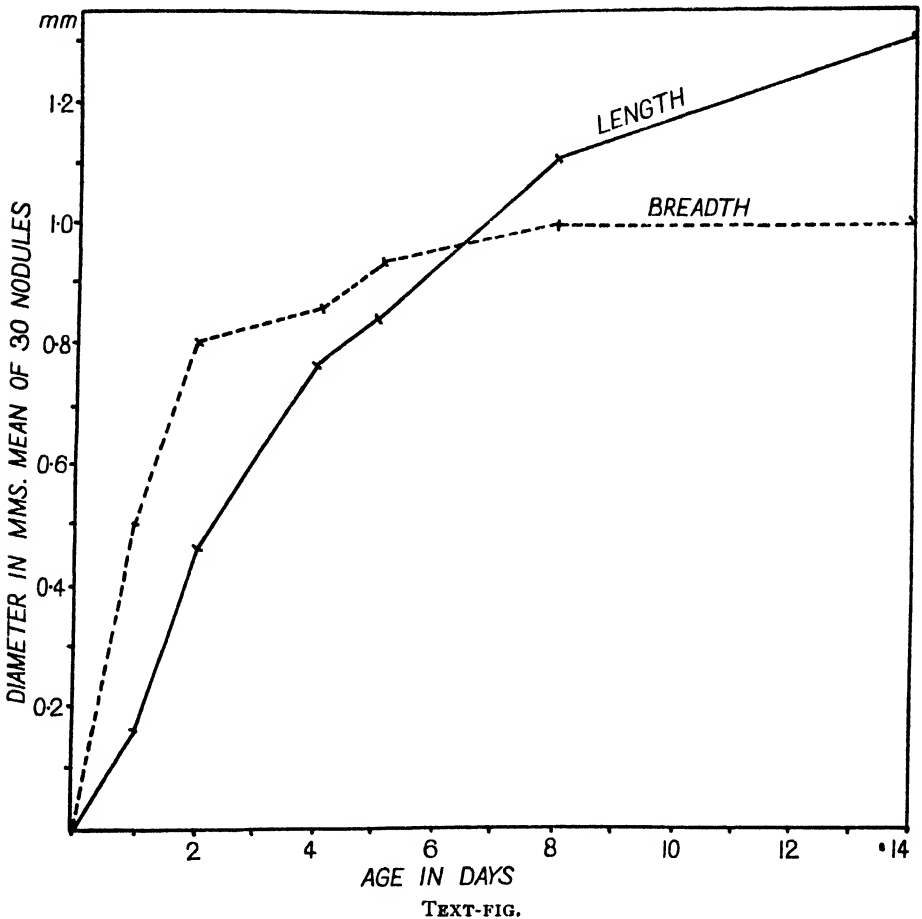
The masses of bacterial zoogloea which are produced as swellings on the young naked infection thread do not as a rule become enclosed by a sheath, but develop into flocculent masses of bacteria, from which individual coccoid rods are released and become scattered in the cytoplasm (Pl. XXII, Figs. 8, and 9). Many of the host cells thus contain scattered

bacteria at an early stage. This method of release is illustrated by Prazmowski (14) and by Dangeard (4). The bacteria are released by a different method in somewhat older cells which contain sheathed infection thread. Blister-like swellings which contain bacteria in the coccoid and short rod stage are formed on the infection thread sheath (Pl. XXII, Fig. 10). The bacteria in them do not appear to be imbedded in a stainable substance as in the tubular portions of the thread. The blisters swell out and eventually burst, releasing the bacteria into the cytoplasm (Pl. XXII, Figs. 11, 12, and 13). Milovidov (9) describes and illustrates similar structures which he calls 'cysts', in the case of *Trifolium* nodules. He states that these cysts remain unbroken until the nodule tissue becomes old, and that from them bacteria are eventually released into the intercellular spaces, where they multiply. In old lucerne nodules, portions of the infection threads persist in the cells, and it is from these that bacteria escape into the middle lamellae of the cell walls and into the intercellular spaces, eventually causing the nodule tissue to disintegrate (19). The cyst-like blisters in lucerne nodules are a means by which the bacteria are released into the young cells.

#### E. FORMATION OF THE BACTEROIDAL TISSUE.

The cells which have ceased to divide swell to about twice their original diameter and become vacuolated. There are at first a number of large vacuoles surrounding a central nucleus, but eventually these run together, producing a large central vacuole, which pushes the nucleus to one side (Pl. XXII, Figs. 14, 15). The latter increases in proportion to the cell, and is at first spherical. The bacteria do not appear to injure either the nucleus or the cytoplasm until the nodule tissue reaches a stage at which disintegration commences. The swelling of the cells commences in the central and proximal region of the nodule, a cap of meristem being left at the distal end. Cell division in this cap causes the nodule to increase in length, so that it becomes cylindrical. This change in proportions takes place when the nodule is about two days old (Text-fig.). An outgrowth of vascular strands from the central cylinder begins at about this time, and follows the course described in detail by Brenchley and Thornton (2) in the case of *Vicia faba*. A rapid multiplication of the bacteria in the swollen cells accompanies this outgrowth. The bacterial cells also increase in size, become banded, and eventually somewhat swollen. They do not usually develop the irregular branching forms which are characteristic of the bacteroidal tissue in many legumes. During the active period of the nodule, these somewhat enlarged banded rods constitute the bulk of the bacterial population of the nodule, the other forms of nodule bacteria being limited to the infection threads and to the region just behind the meristem cap where continuous

infection of the new cells takes place. The banded rods are thus presumably the stage which is of chief importance in fixing nitrogen.



The changes which take place as the nodule ages and which bring about its decay are described elsewhere (19).

#### SUMMARY AND ABSTRACT.

1. The bacteria infect the root hairs, the 'infection threads' passing into the cortex without invading the central cylinder of the root. Cell division is induced, forming a round mass of meristem cells into which the infection threads enter.

2. The infection threads are naked at their growing points and tend to swell into zoogloal masses in the cells. A sheath continuous with the wall of the host-cell is formed round the infection threads in their older portions.

3. The zoogloal masses do not become surrounded by a sheath, but

release bacteria into the host cytoplasm. At a later stage, blister-like swellings on the infection thread sheath develop, and, by their rupture, release more bacteria into the cytoplasm.

4. Dividing cells containing young infection threads occur, but division of the host-cells ceases by the time the infection thread sheath is formed. Swelling of the host-cells and multiplication of the bacteria in the cytoplasm produces the 'bacteroidal tissue' in which the bacteria become somewhat swollen banded rods. The host-cells are apparently uninjured by the bacteria save in old nodule tissue.

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#### EXPLANATION OF PLATES XXI AND XXII.

Illustrating Mr. H. G. Thornton's paper on the Early Development of the Root Nodule of Lucerne.

##### PLATE XXI.

Fig. 1. Root-hair containing infection thread (fresh material  $\times 1,000$ ). *l.*, curled tip; *if.*, infection thread.

Fig. 2. Section of very young nodule (camera lucida drawing  $\times 1,000$ ). *x.*, xylem of root; *m.*, meristematic cells of cortex; *if.*, infection thread; *z.*, bacterial zoogloea.

Fig. 3. Section of young nodule ( $\times 500$ ). *c.*, cortex; *m.*, meristematic tissue; *x.*, xylem of root.

Fig. 4. Infection of a dividing cell (camera lucida drawing  $\times 1,000$ ). *if.*, infection thread; *z.*, bacterial zoogloea; *m.*, dividing nucleus.

Fig. 5. Infection thread, *if.*, without sheath, passing through three cells.  $\times 1,000$ .

Fig. 6. Older infection thread with sheath, *s.*  $\times 1,000$ .

Fig. 7. Infection threads, *s.*, with sheaths and masses of bacterial zoogloea, *z.*  $\times 1,000$ .

#### PLATE XXII.

Fig. 8. Bacteria passing out from bacterial zoogloea, *z.* *st.*, infection thread with sheath (camera lucida drawing,  $\times 1,000$ ).

Fig. 9. Formation of blister, *b.*, on infection thread with sheath, *st.* *bac.*, bacteria previously released into the cytoplasm (camera lucida drawing,  $\times 1,000$ ).

Fig. 10. Swelling of blisters, *b.* *st.*, infection threads (camera lucida drawing,  $\times 1,000$ ).

Fig. 11. Breaking of blisters, *b.*, and release of contained bacteria (camera lucida drawing,  $\times 1,000$ ).

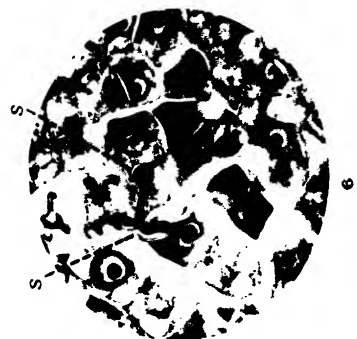
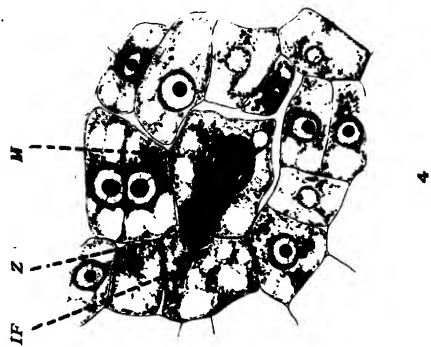
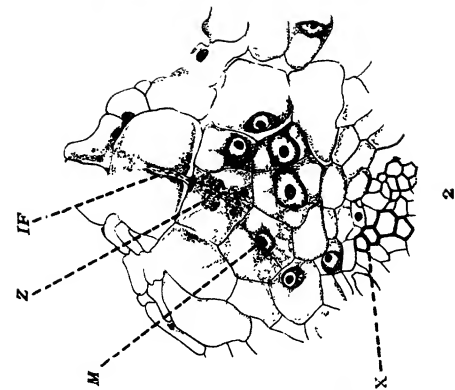
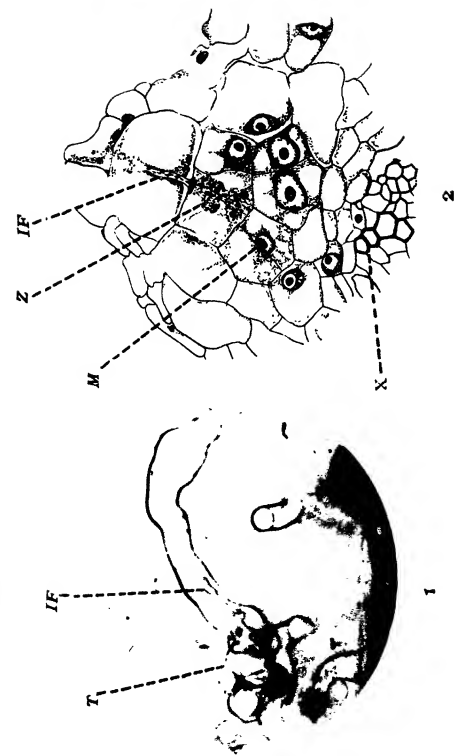
Fig. 12. Infection thread with sheath, *if.*, forming blister, *b.*  $\times 1,000$ .

Fig. 13. Infection thread, *if.*, with blister, *b.*, indenting the nucleus of the host-cell, *n.*  $\times 1,000$ .

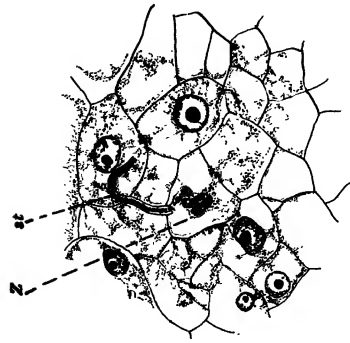
Fig. 14. Young bacteroidal tissue showing vacuoles, *v.*, surrounding host-cell nuclei, *n.*  $\times 1,000$ .

Fig. 15. Fully developed bacteroidal tissue showing central vacuoles, *v.*, displacing the host-cell nuclei.  $\times 1,000$ .

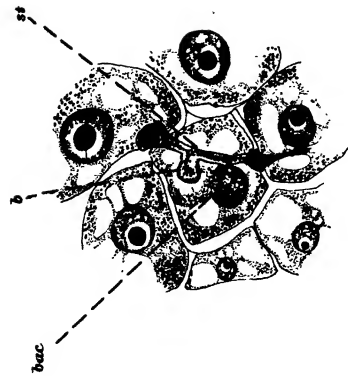




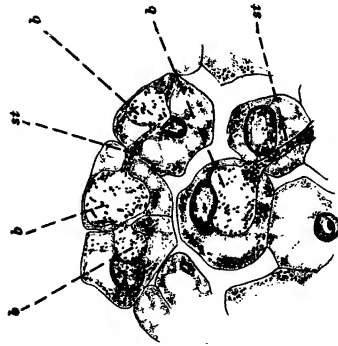




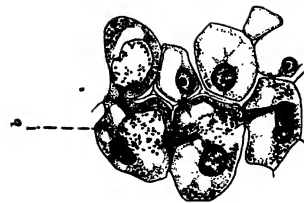
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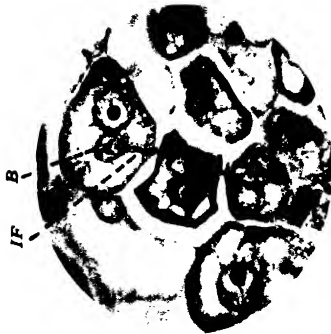
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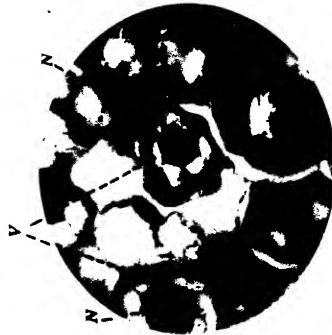
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Hub, London



## ON THE INFLUENCE OF SOIL TEMPERATURE ON THE GERMINATION INTERVAL OF CROPS.

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THE data collected under the Agricultural Meteorological Scheme of the Ministry of Agriculture have provided information for a number of years in a number of places on the dates of sowing and appearance above ground of wheat, winter oats, spring oats, spring barley, turnips and swedes.

The varieties reported on varied from place to place and year to year, but a study of the variation showed that in all except a few cases there was no varietal difference in "germination interval" as measured by the interval between sowing and appearance above ground.

The varieties which were anomalous in respect of germination interval have been omitted from this investigation<sup>1</sup>.

<sup>1</sup> For each crop in each year, the variance in interval between sowing and appearance above ground was analysed into portions due to differences between varieties and differences within varieties. Of twenty-three cases so examined there were fifteen in which there was no significant difference between the variances within and between varieties, four cases in which the variance between varieties was significantly *lower* than that within, and four in which it was significantly higher. The four cases in which the variance between varieties was significantly higher were

(1) *Winter oats*, 1924-5. Here an examination showed that the higher variance between varieties was due to one variety, Potato—Victory grown at Craibstone only.

This variety gave an interval of 15 days between sowing and appearance above ground, the mean value for all the other varieties being between 20 and 24 days.

(2) *Turnips*, 1924-5. Here two varieties, Aberdeen Green Top and Aberdeen Bullock Yellow, gave a mean interval of 6 or 7 days and the other two, Favourite Purple and Top Aberdeen, grown at Cockle Park only, gave an interval of 24 days, an undoubtedly significant difference.

(3) *Spring oats*, 1927. Here an examination showed that the higher variance between varieties was due to two varieties, Svålof Victory and Swedish King, grown at Rothamsted only. For these two the intervals were respectively 35 and 38 days, all the others being between 14 and 26 days.

(4) *Swedes*, 1928. Here out of ten varietal means all were between 10 and 14 days, except Lord Derby grown only at Aber, Model grown only at Wye and Caledonian grown only at Cockle Park. These gave intervals of 6, 7 and 17 days respectively.

Of these Model and Caledonian were grown in 1927 and 1926 when the interval was not outstandingly different from the average.

There was almost certainly a tendency for reporters to report different varieties sown

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In some stations since 1925 and in all stations since 1926 4 in. and 8 in. soil temperatures have been observed.

Information was therefore available for a study of the relation between soil temperature and germination interval. Tables I-VI show the stations and varieties which have been used in this investigation.

Table I. *Winter wheat.*

Station	Year		
	1924-5	1926-7	1927-8
Craibstone ...	—	Standard Red (3 sowings)	Standard Red (3 sowings)
Sprowston ...	—	Cambridge Browick Yeoman II Squareheads Master Setter Fox Weibull's Standard Wilhelmina Iron III Little Joss	Yeoman II Wilhelmina Iron III Squareheads Master
Rothamsted ...	Standard Red	Million III Standard Red	Yeoman II Swedish Iron Million III Squareheads Master
Good Easter ...	—	Squarehead Master Yeoman II Little Joss Cambridge Browick Fox Wilhelmina Weibull's Standard Iron III	Yeoman II Wilhelmina Iron III Squareheads Master
Sutton Bonington ...	—	Yeoman I Little Joss Fox Crown	Yeoman I Fox Little Joss Crown
Wye ...	—	Yeoman II Yeoman I	Yeoman I Renown

in the same place on the same day as appearing above ground on the same day, whether this was strictly true or not, and this is very probably the explanation of the four cases in which the variance between varieties was significantly lower than that within; it is probable also that for this reason the variance between varieties is too low in other cases. But, although the variance is too low, there is no reason to suppose that the mean values are biased and this is the justification of the use of the data for the present purpose.

So as to maintain the apparent homogeneity of the material, the varieties which caused the varietal differences in cases (1), (2), (3) and (4) just mentioned have been omitted from the investigation.

This would in any case have been necessary in cases (1) and (2) because the corresponding soil temperature data were not available. For turnips there are in any case insufficient data for working out correlations.

Table I (*contd.*)

		Year		
Station		1924-5	1926-7	1927-8
Long Sutton	...	—	Squareheads Master Yeoman II Little Joss Cambridge Browick Fox Wilhelmina Weibull's Standard Iron III	Yeoman II Wilhelmina Iron III Squareheads Master
Aberystwyth	...	—	Standard Red (2 sowings)	—
Newton Abbot	...	—	Yeoman II Iron III Wilhelmina Cambridge Browick	—

Table II. *Winter oats.*

		Year			
Station		1924-5	1925-6	1926-7	1927-8
Craibstone	... ..	—	—	Grey Winter (3 sowings)	Grey Winter (3 sowings)
Sprowston	... ..	—	—	Grey Winter Marvellous Bountiful No. 914 Black Winter	Plentiful Grey Winter Marvellous Bountiful
Rothamsted	... ..	Grey Winter	—	Black Winter Grey Winter	—
Good Easter	... ..	—	Bountiful Black Winter Marvellous Grey Winter	Grey Winter Bountiful Marvellous No. 914 Black Winter Victory	Plentiful Grey Winter Marvellous Bountiful
Sutton Bonington	... ..	—	—	Bountiful Black Winter Marvellous White Winter Grey Winter	—
Wye	... ..	—	—	Bountiful	—
Long Sutton	... ..	—	—	Bountiful Marvellous No. 914 Grey Winter Black Winter	—
Newton Abbot	... ..	—	Bountiful Black Winter Marvellous Grey Winter	—	—

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Table III. *Spring oats.*

Station	Year			
	1925	1926	1927	1928
Craibstone... ..	—	Victory (2 sowings) Potato (2 sowings)	Potato (3 sowings) Victory (3 sowings)	Potato (2 sowings) Victory (2 sowings)
Sprowston ... ..	—	Golden Rain Victory Abundance Black Tartarian A. 88	Black Potato Thousand Dollar Abundance White Cross Victory Golden Rain A. 88	Victory Thousand Dollar Golden Rain A. 88
Rothamsted ... ..	Giant Eliza	—	—	—
Good Easter ... ..	—	Abundance Victory Golden Rain Black Tartarian	Golden Rain Thousand Dollar Abundance Black Potato White Cross Victory	Abundance Golden Rain Victory Thousand Dollar A. 88
Sutton Bonington	—	—	Abundance Cropwell Victory	Victory Abundance Cropwell Ascot
Sandford ... ..	—	—	King	—
Wye ... ..	—	Victory	Marvellous	Marvellous
Long Sutton	—	—	Abundance Victory Golden Rain White Cross Thousand Dollar Black Potato A. 88	Golden Rain Victory Thousand Dollar A. 88
Aber ... ..	—	—	—	Goldfinder Victory Record Black Tartarian
Aberystwyth	—	Record (2 sowings)	—	—
Newton Abbot ... ..	—	Abundance Thousand Dollar White Cross Black Potato Black Tartarian A. 88 Victory Golden Rain	Golden Rain Thousand Dollar Victory A. 88	—

Table IV. *Spring barley.*

Station	Year			
	1925	1926	1927	1928
Craibstone ... ..	—	Plumage Archer (2 sowings)	Plumage Archer (3 sowings)	Plumage Archer (4 sowings)
Sprowston ... ..	—	Plumage Archer Sunrise Spratt Archer Archer Goldthorpe Beaven's No. 25 No. 824 No. 825 New Cross Beaven's Archer	Spratt Archer New Cross Archer Goldthorpe No. 825 Beaven's No. 25 Sunrise Beaven's Archer No. 824	Spratt Archer No. 824 No. 825 Plumage Archer
Rothamsted	Plumage Archer	—	—	Standwell
Good Easter ... ..	—	Plumage Archer Beaven's Archer Sunrise Spratt Archer Archer Goldthorpe Beaven's No. 25 No. 833 No. 832	No. 824 No. 825 Archer Goldthorpe Beaven's No. 25 Spratt Archer Plumage Archer, 1924 Beaven's Archer Webb's Sunrise	Plumage Archer Spratt Archer No. 824 No. 825
Sutton Bonington ...	—	—	Spratt Archer Plumage Archer Beaven's 1924 Burton Malting Gold New Cross Triumphant	Spratt Archer New Cross Plumage Archer Sunrise
Wye 6 ... ..	—	Plumage Archer	Plumage Archer Spratt Archer	Plumage Archer (2 sowings)
Long Sutton ... ..	—	—	—	Spratt Archer Plumage Archer No. 824 No. 825
Newton Abbot ... ..	4	—	Beaven's Archer Webb's Sunrise Spratt Archer Archer Goldthorpe	—

Table V. *Turnips.*

Station	Year		
	1926	1927	1928
Craibstone ... ..	Glenlogie	—	Lettyton Green Top (2 sowings)
Wye ... ..	—	White	—

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Table VI. *Swedes.*

Station	Year		
	1926	1927	1928
Craibstone ...	Inverquhomery	—	Aberdeenshire Prize
Sprowston ...	Best-of-all	Giant King	Best-of-all
	Smith's Green Top	Superlative	
	Purdy's Purple Top	Gateacre	
	Gateacre	Conqueror	
	Superlative	Smith's Green Top	
	Giant King	Purdy's Purple Top	
Good Easter ...	Conqueror	Best-of-all	—
	Best-of-all	Gateacre	
	Giant King	Superlative	
	Conqueror	Best-of-all	
	Smith's Green Top	Conqueror	
	Superlative	Giant King	
Long Sutton ...	Gateacre	Smith's Green Top	Best-of-all Giant King Superlative Gateacre Conqueror
	Best-of-all	Best-of-all	
	Conqueror	Giant King	
	Superlative	Conqueror	
	Smith's Green Top	Smith's Green Top	
	Gateacre	Superlative	
Wye ...	Giant King	Gateacre	
	Superlative	Model	—

Table VII. *Wheat.*

Correlation data between "germination interval" and mean daily soil temperature.

Station	Year	Interval (days)	Mean soil temperature (°F.)	
			4 in.	8 in.
Craibstone ...	1926-7	10.00	56.83	55.92
	"	26.00	41.96	43.41
	"	41.00	38.00	38.33
	1927-8	6.00	57.70	56.68
	"	6.00	45.96	46.27
	"	34.00	38.01	39.22
Sprowston ...	1926-7	29.33	42.10	42.82
	1927-8	30.00	42.82	43.42
Rothamsted ...	1924-5	25.00	42.72	—
	1926-7	26.50	45.30	46.14
	1927-8	16.00	51.38	50.99
Good Easter ...	1926-7	9.12	43.61	45.15
	1927-8	56.00	39.12	40.98
Sutton Bonington ...	1926-7	38.75	39.89	40.62
	1927-8	17.75	40.70	41.22
Wye ...	1926-7	18.50	46.29	46.39
	1927-8	11.00	51.38	51.02
	"	24.00	42.45	43.19
	1926-7	31.00	43.77	44.12
Long Sutton ...	1927-8	33.00	41.15	42.06
	1926-7	29.00	43.10	44.42
Aberystwyth ...	"	27.00	41.79	42.65
	1926-7	33.00	39.95	40.32



Table VIII. *Winter oats.*

Correlation data between "germination interval" and mean daily soil temperature.

Station	Year	Interval (days)	Mean soil temperature (° F.)	
			4 in.	8 in.
Craibstone ... ..	1926-7	11.00	56.56	55.74
	"	26.00	41.96	43.41
	"	44.00	37.82	38.19
	1927-8	8.00	57.13	56.21
	"	13.00	47.67	47.65
Sprowston ... ..	"	40.00	38.25	39.38
	1926-7	37.80	40.92	41.68
	1927-8	50.75	38.98	39.64
Rothamsted ... ..	1924-5	14.00	52.19	—
	1926-7	32.50	43.27	44.14
Good Easter ... ..	1925-6	62.00	37.97	39.15
	1926-7	28.50	44.26	45.51
	1927-8	55.00	39.08	40.90
Sutton Bonington ... ..	1926-7	33.20	41.29	42.04
Wye ... ..	1926-7	24.00	45.13	45.53
Long Sutton ... ..	1926-7	29.20	43.48	43.99
Newton Abbot ... ..	1925-6	40.00	37.91	38.53

Table IX. *Spring oats.*

Correlation data between "germination interval" and mean daily soil temperature.

Station	Year	Interval (days)	Mean soil temperature (° F.)	
			4 in.	8 in.
Craibstone ... ..	1926	26.00	40.78	40.34
	"	17.00	45.90	44.89
	1927	21.00	41.85	41.05
	1928	25.00	40.98	39.82
	"	26.00	40.72	39.62
Sprowston ... ..	1926	13.60	48.98	47.51
	1927	22.14	43.84	43.50
	1928	30.50	40.55	40.58
Rothamsted ... ..	1925	35.00	38.98	—
Good Easter ... ..	1926	23.00	44.14	43.58
	1927	19.67	45.95	45.23
	1928	28.00	43.33	42.78
Sutton Bonington ... ..	1927	24.00	44.05	43.47
	1928	27.00	42.76	42.11
Sandford ... ..	1927	14.00	53.49	51.34
Wye ... ..	1926	22.00	42.89	43.26
	1927	21.00	43.79	43.51
	1928	26.00	40.99	40.81
Long Sutton ... ..	1927	13.71	51.15	50.62
	1928	19.25	47.37	46.72
Aber ... ..	1928	10.00	55.68	53.29
Aberystwyth ... ..	1926	20.00	44.09	43.73
Newton Abbot ... ..	1926	17.62	51.48	50.35
	1927	20.50	47.37	46.61

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Table X. *Spring barley.*

Correlation data between "germination interval" and mean daily soil temperature.

Station	Year	Interval (days)	Mean soil temperature (° F.)	
			4 in.	8 in.
Craibstone ... ..	1926	24-00	40-76	40-38
	"	16-00	46-04	44-97
	1927	19-33	41-86	41-04
	1928	17-75	42-78	41-57
Sprowston ... ..	1926	11-78	49-11	47-61
	1927	14-00	48-64	47-37
	1928	27-00	42-19	41-82
Rothamsted ... ..	1925	15-00	39-28	—
	1928	12-00	46-11	44-53
Good Easter ... ..	1926	22-75	44-36	43-70
	1927	19-37	46-12	45-37
	1928	23-00	46-14	45-21
Sutton Bonington ... ..	1927	18-57	45-17	44-51
	1928	19-00	45-78	44-90
Wye ... ..	1926	18-00	43-84	43-60
	1927	17-00	45-80	44-57
	"	10-00	52-60	49-57
	1928	22-50	41-46	41-12
Long Sutton ... ..	1928	18-00	47-35	46-59
Newton Abbot ... ..	1927	20-00	47-17	46-47

Table XI. *Swedes.*

Correlation data between "germination interval" and mean daily soil temperature.

Station	Year	Interval (days)	Mean soil temperature (° F.)	
			4 in.	8 in.
Craibstone ... ..	1926	15	46-19	45-10
	1928	10	46-82	45-93
Sprowston ... ..	1926	5	56-82	53-92
	1927	15	59-04	56-64
	1928	10	58-01	55-42
Good Easter ... ..	1926	9	58-75	55-27
	1927	15	58-71	56-19
Long Sutton ... ..	1926	6	62-53	60-21
	1927	26	60-68	59-46
	1928	13	59-68	58-83
Wye ... ..	1926	7	59-30	57-48
	1927	8	59-20	57-08

The convention was adopted that intervals which did not overlap were counted separately, but where they overlapped a mean interval was calculated. The mean soil temperature used was the mean of the 9 hr., 15 hr., 21 hr. readings. Where intervals overlapped an average of the daily mean temperature was calculated by totalling all the daily temperatures in the overlapping intervals and dividing by their number.

By this means enough pairs of observations to correlate were obtained. The data used are shown in Tables VII–XI. It will be seen that the number of pairs of observations varies from 12 to 24 for the different crops. (For turnips there were only four pairs of observations available and these were not sufficient in number to correlate.)

Both the regression of “germination interval” on 4 in. and 8 in. soil temperatures separately and the correlations were worked out. The former is naturally the more fundamental and useful for this purpose, as it gives the average decrease in “germination interval” for each degree increase in soil temperature.

On the basis of the data in Tables VII–XI we have the following results for mean interval, mean 4 in. and 8 in. soil temperatures and the variabilities of these quantities.

Table XII. *Means.*

Crop		Mean interval (days)	Standard error	Mean 4 in. soil		Mean 8 in. soil	
				temp. (° F.)	Standard error	temp. (° F.)	Standard error
Winter wheat	...	25.13	2.55	44.17	1.12	44.79	1.05
Winter oats	...	32.29	3.79	43.76	1.52	43.86	1.37
Spring oats	...	21.75	1.19	45.05	0.90	44.55	0.81
Spring barley	...	18.25	0.98	45.13	0.71	44.47	0.57
Swedes	...	11.58	1.66	57.14	1.49	55.13	1.40

Table XIII. *Variabilities.*

Crop				s.d.* of interval (days)	s.d. of 4 in. soil temp. (° F.)	s.d. of 8 in. soil temp. (° F.)
Winter wheat	...	...	...	12.23	5.39	4.93
Winter oats	...	...	...	15.63	6.26	5.49
Spring oats	...	...	...	5.81	4.41	3.89
Spring barley	...	...	...	4.36	3.18	2.48
Swedes	...	...	...	5.76	5.16	4.84

\* Calculated from  $\sqrt{\frac{S(x-x)^2}{n-1}}$ .

The standard errors do not seem unduly large when we realise that they include sources of variation due not only to varietal but also to soil and place differences.

The mean intervals for the two winter cereals do not differ significantly, while for the two spring cereals the difference is just significant. The mean interval for swedes is again significantly different from that of the other crops.

The mean soil temperatures for all the crops except swedes do not

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differ significantly from one another. Nor are the mean 4 in. and 8 in. soil temperatures significantly different.

When we examine the variabilities by Fisher's "z" test we find that the variabilities of interval for the two winter-sown crops are not significantly different, nor are those for the spring cereals and swedes, but the last three differ significantly from the first two. The variability of 8 in. soil temperature for the spring barley is perhaps significantly smaller than for the other crops, apart from this soil temperatures exhibit no significant differences in variability.

The regressions and correlations are as follows:

Table XIV.

Crop	Regression of "germination interval" on soil temperature (days per ° F.)				Correlation of "germination interval" and soil temperature (° F.)	
	4 in.	Standard error	8 in.	Standard error	4 in.	8 in.
Winter wheat ...	-1.69	0.33	-1.89	0.36	-0.75	-0.74
Winter oats ...	-2.18	0.31	-2.40	0.39	-0.87	-0.86
Spring oats ...	-1.17	0.13	-1.17	0.14	-0.89	-0.88
Spring barley ...	-0.81	0.26	-1.21	0.31	-0.59	-0.68
Swedes... ..	-0.02	0.35	+0.07	0.38	-0.02	+0.06

Neither the regressions nor the correlations of any one crop with 4 in. and 8 in. soil temperatures differ significantly from one another.

For all the cereal crops the correlations are significant and, except for Spring Barley, high. The results for the winter-sown cereals are different from the spring-sown and we may summarise them by saying that the "germination interval" for winter wheat and oats is shortened by from 1.5 to 2 days for each increase of a degree F. in 4 in. or 8 in. soil temperature; for spring cereals the corresponding shortening is about a day.

Swedes clearly exhibit no correlation between "germination interval" and soil temperature.

This may well be because not soil temperature but soil moisture is the limiting factor.

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# STUDIES IN SAMPLING TECHNIQUE: CEREAL EXPERIMENTS.

## I. FIELD TECHNIQUE.

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(With One Text-figure.)

PRELIMINARY experiments carried out in the summer of 1928 had indicated that sampling methods might be used to obtain a satisfactorily precise estimate of the yield of small cereal plots<sup>(1)</sup>. It was thought desirable to test the method more thoroughly, and to compare the estimates with figures obtained by large-scale methods. Accordingly samples were taken from all the plots of the three Rothamsted cereal experiments of 1928, the plots later being harvested with a binder in the ordinary manner. Samples were also taken from the 16 plots of a small experiment on barley at Wellingore, Lincs., but no direct measurement of total yield was made for these plots. There were 210 plots in all, but of these 50 were sampled in four sections, corresponding with minor differences in manurial treatment; so that the total number of plots dealt with amounted to 360. The large-scale method, however, treated each of the 50 as a single plot, and thus provided only 210 yield figures.

### THE METHOD.

The sampling method was Method (a) of the earlier paper on cereals<sup>(1)</sup>: 20–32 metre-lengths of drill were cut at randomly located points in each plot, with the restriction that half the number should be cut from each half of the plot; or, in the case of the 50 Rothamsted barley plots, one-quarter of the number from each of the four sections into which the plots were divided. Since the metre-lengths, apart from this restriction, were located independently, they represent the constituent “sampling-units” of the sample<sup>(2)</sup>.

### NUMBER OF SAMPLING-UNITS.

The earlier work had shown that 30 metre-lengths of drill from a plot 1/40th acre in area, might be expected to give a yield estimate with a standard error of about 5 per cent. This figure would vary comparatively



little with the size of the plot over a considerable range, unless the field were very heterogeneous; as the area increased, slightly larger samples would be required to give the same accuracy. It was therefore decided to take 30 metre-lengths from the 48 plots of the oats experiment, whose area was  $1/40$ th acre; and 8 from each quarter, or 32 in all, from each of the 50 plots of the barley experiment, where the area was again  $1/40$ th acre. The 96 plots of the wheat experiment were only of about  $1/55$ th acre, and it was considered sufficient to take only 24 metre-lengths from each.

It will be noticed that only 8 metre-lengths were taken from each quarter of the barley plots, whereas to get a yield estimate with standard error as low as 5 per cent. a considerably larger number would have been necessary. The reason for this apparent change of standard is best seen from a consideration of the plan of the experiment. There were two  $5 \times 5$  Latin squares, giving 50 plots of  $1/40$ th acre. In one of the squares the treatments were:

- |                        |                                   |
|------------------------|-----------------------------------|
| 1. No nitrogen         | } To give 0.2 cwt. of N per acre. |
| 2. Sulphate of ammonia |                                   |
| 3. Muriate of ammonia  |                                   |
| 4. Cyanamide           |                                   |
| 5. Nitrate of soda     |                                   |

In the second square urea replaced "no nitrogen," and the dressings were at the rate of 0.4 cwt. of N per acre.

The arrangement of the plots in Latin squares ensured that each treatment should occur on five different plots.

Now one quarter of each plot received no further treatment; a second quarter received muriate of potash at the rate of 0.5 cwt.  $K_2O$  per acre; a third superphosphate at the rate of 0.6 cwt. of  $P_2O_5$  per acre; and a fourth both these treatments. Then the direct comparison of the effects of potash and phosphate is made between means of 100 quarter-plots; while interactions between these manures and the various nitrogenous treatments will be tested on means of 20 quarter-plots (except with "no nitrogen" and "urea," where there are only 10). These numbers are halved where the interactions between potash and phosphate are being studied, or where the two levels of nitrogenous manuring are treated separately.

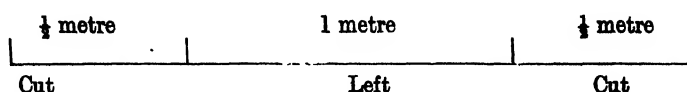
Now since these large numbers of quarter-plots contribute to the various comparisons, individual estimates of yield from the quarter-plots need not be found with so high a degree of accuracy as from the whole

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plots. It was consequently considered sufficient to take only 8 metre-lengths from each. The justification for this lowering of the standard of accuracy will be seen in the results.

#### STRUCTURE OF SAMPLING-UNITS.

The sampling-unit, as has already been stated, was a metre-length of drill. The constituent half-metres were not, however, contiguous, but were separated by an interval of one metre. The measuring-rod was thus of the form shown in the figure. Horizontally projecting nails marked the ends of the half-metre-lengths which were to be cut.



The advantage gained by this division of each sampling-unit into two separated units lies in the increased representativeness of the resulting sample, and can be measured directly by appropriate statistical methods, provided that the weights of produce from the individual units are recorded. This was not done in the present series of experiments, but had previously been done on a number of occasions, the results then obtained justifying the method. The statistical procedure is an analysis of variance, and consists in comparing the variation *between* whole-metre-lengths with that between their constituent half-metre-lengths: that is, with that *within* metre-lengths. If the former is the greater to an extent, which would not often occur merely by chance, it is concluded that more information about the crop would have been obtained had the half-metres been completely scattered, instead of being grouped in closely associated pairs. If on the other hand the two variations do not differ significantly, it is concluded that no information has been lost by the association.

The actual test experiments consisted in comparing the variation between and within metre-lengths; first, where constituent half-metres were immediately contiguous; and secondly, where an interval separated them. The measure of variation employed is R. A. Fisher's "Mean Square," and comparisons are effected by means of the "z" test (3).

As an example, the analysis is given for counts of shoot-number made on June 29th, 1928. 32 metre-lengths of drill were selected at random from a small plot of wheat, and separate counts were made of the shoots in each half-metre-length. There were thus 64 observations in all, and the 63 degrees of freedom were divided into 31 for the differences between

whole-metre lengths, and 32 for differences between the constituent half-metre-lengths of the same metre-length.

	Degrees of freedom	Sum of squares	Mean square	$z$
Between metre-lengths	31	1733.7	55.93	—
Within metre-lengths	32	725.5	22.67	0.4514
Total	63	2459.2	—	—

The 5 per cent. point of  $z$  is 0.2361, so that mean square "Between metre-lengths" is significantly greater than that "Within metre-lengths," and there is a loss of information as compared with that obtainable from the same number (64) of independently located half-metres.

On July 5, 1928, counts of ear-number were made on the same wheat, but using a dissected 4-ft.-length instead of a metre-length of drill as the observational unit (Fig. 1).

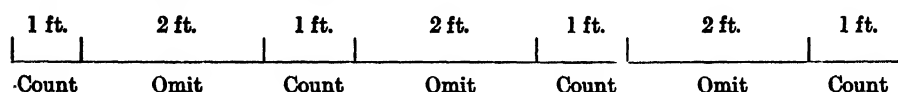


Fig. 1.

	Degrees of freedom	Sum of squares	Mean square	$z$
Between 4-ft.-lengths	16	361.37	22.59	—
Within 4-ft.-lengths (= between 1-ft.-lengths in the same 4-ft.-length)	96	1811.75	18.87	0.0900

Here the 5 per cent. point of  $z$  is 0.2763, and 1-ft.-lengths within the same 4-ft.-length do not resemble each other appreciably more than do 1-ft.-lengths from different 4-ft.-lengths. There is thus no loss of information when the increased labour of completely independent location is avoided.

A similar result was obtained when the sampling-unit consisted of two half-metres separated by a metre, and this pattern was accordingly adopted.

#### LOCATION OF SAMPLING-UNITS.

In determining the points at which sampling-units were to be cut, two numbers were chosen, one representing a drill-row, and the other a distance along the plot in paces. If there were  $n$  drill-rows in a plot, and 30 sampling-units were required from each plot, 30 numbers from 1 to  $(n - 2)$  were chosen at random, by the use of Tippet's "Tables of Random Sampling Numbers" (5). To each of these was added 1, so that there were 30 numbers ranging from 2 to  $(n - 1)$ , representing 30 independent selections of a row other than an edge-row (the number of the

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row being counted from the edge-row for convenience). Now to each of the first 15 there was assigned at random a number from 1 to  $(m - 1)$  and to each of the second 15 a number from 0 to  $(m - 2)$ , where  $m$  is the length of a half-plot, in paces. This ensured that no crop should be cut within 1 pace from the ends of the plot. The first 15 pairs of numbers were then allocated to one half-plot, and the remaining 15 to the other half-plot. The procedure in the field was then to start at the end of the plot and walk the required number of paces down a certain row, and to place the measuring-rod with its end just touching the toe of the forward foot. To minimise trampling of the crop, the pairs of numbers for each half-plot were arranged in ascending order of paces along the plot, so that there was steady progress in this direction, although it was still necessary to cross from one row to another. When the 15 metre-lengths had been taken from one half-plot, the counting of paces for the second set of 15 was started from the middle of the plot—*i.e.* at the boundary between the half-plots.

#### HARVESTING OF THE SAMPLING-UNITS.

The produce of each sampling-unit was cut about an inch above the ground with large scissors, and the two half-metre-lengths tied together into a single sheaflet. Before tying the heads were thrust into a perforated paper bag, and this was secured by means of the string of a label which indicated the plot and the serial number of the sampling-unit. It was found unnecessary to tie at any other point since the bag covered about a third of the length of the sheaflet. The sheaflets from a plot were tied into a single sheaf, which was suspended from the roof of a well-ventilated room.

The paper bags were 7-lb. sugar bags, as supplied to grocers, and were of fairly thick and strong yellow paper, glazed externally. Thirty-two holes, just small enough to prevent a cereal grain from passing, were punched in each bag, to ensure rapid drying of the heads.

A few samples were found to be mouldy when examined before threshing, but these were in all cases very weedy, and were probably cut when the corn was slightly damp. It is expected that with an increase in the number of perforations, and more care in cutting only when the crop is quite dry, there will be no trouble from this cause.

## WEIGHING AND THRESHING.

The sheaflets were weighed before threshing, and the grain after threshing, the difference, corrected for the weight of the bag, string and label, being taken as straw. The balances were supplied by Messrs W. and T. Avery, of Birmingham, and were direct-reading machines with charts graduated from 0 to 100 gm. at intervals of 1 gm. An adjustable air-damping device made weighing a very rapid process, since the pointer was almost dead beat.

The electrically driven bench thresher and winnower is described in the next paper.

## LABOUR.

Table I compares the labour expended per plot in harvesting the three Rothamsted experiments by large-scale and by sampling methods respectively. The large-scale methods consisted in cutting with a binder, stooking, carting to the threshing machine, threshing, and weighing the produce. Time spent in supervising these operations is also included in the estimates. It must be borne in mind, however, that the exceptionally dry summer of 1929 made it possible to thresh all experimental produce in the field, the labour of stacking being entirely saved and that of carting much reduced in consequence. It is estimated that the large-scale figures would be almost doubled in a normal season.

Table I. *Man-hours per plot.*

	Large-scale method	Sampling method
1. Oats experiment	1.67	2.25
2. Wheat experiment	1.69	1.95
3. Barley experiment	2.18	2.36

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## STUDIES IN SAMPLING TECHNIQUE: CEREAL EXPERIMENTS.

### II. A SMALL-SCALE THRESHING AND WINNOWER MACHINE.

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(With Plate II and Two Text-figures.)

ON the adoption of a sampling method for the estimation of the yield of small experimental plots, the need arose for a threshing machine capable of threshing quickly and accurately the small sampling-units which comprise the sample from an individual plot. These sampling-units contain various numbers of ears from some three or four to twenty or more, and there is no commercial machine which deals adequately with such small quantities of grain. The number of sampling-units from a typical randomised block experiment may amount to 2500, and to thresh and winnow such numbers in two distinct operations would take no inconsiderable time. As a direct result of this, a machine was constructed in which these two operations were successively performed, without the need of handling the intermediate products. It is the object of this paper to describe this machine and the manner in which it was used for this purpose at Rothamsted last season. Wheat, oats and barley were treated, only minor alterations of the size of the screens, the speed of rotation and the strength of the blast for the separation of the chaff being necessary.

The machine depicted in Fig. 1, in its essentials like the ordinary commercial machine, consists of two main parts, the actual beating arrangement shown in the lower photograph, and a reciprocating screening attachment shown in vertical section in Fig. 2. The overall length of the completed thresher is some 3 ft., its breadth is about 10 in., and the height about 4 ft.

The ears, on the straw, are fed into the machine by the door at *A*, long straw and the fraction often styled "pulse" in large-scale operations are ejected at *B*, while the chaff is blown out at *C*. The dressed grain is collected in the small drawer at *D*. With suitable adjustments of the

speed of rotation of the drum, and of the size of the screens, and their rate of reciprocation, coupled with a favourable blast from the blower at *E*, the sample of dressed grain is practically free from extraneous matter. The most difficult cereal to thresh and winnow clean was found to be barley, some trouble in removing all the awns being experienced.

The actual separation of the grain from the ear is effected by the revolving toothed cylinder, rotating at fairly high speeds in a similarly toothed concave shown in the lower photograph (Pl. II)<sup>1</sup>. The distance

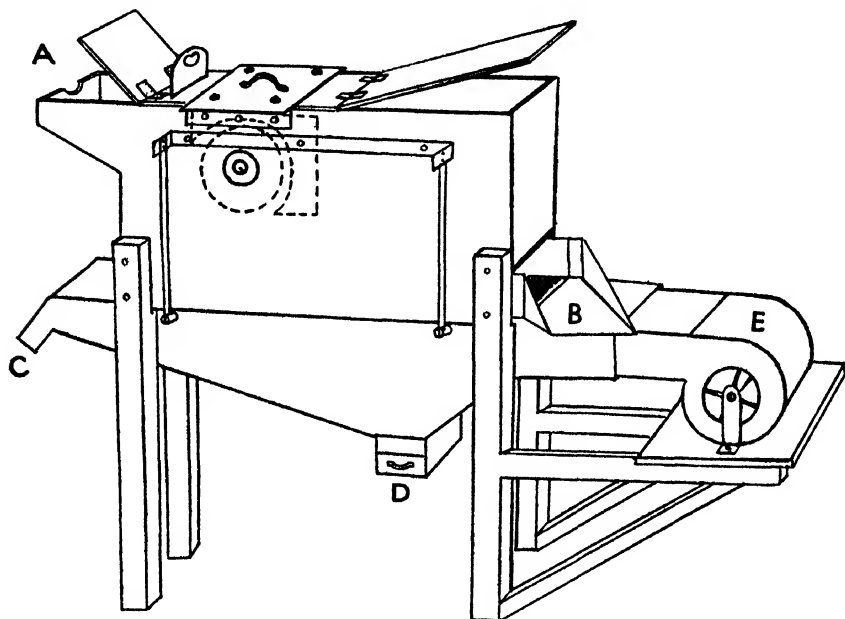
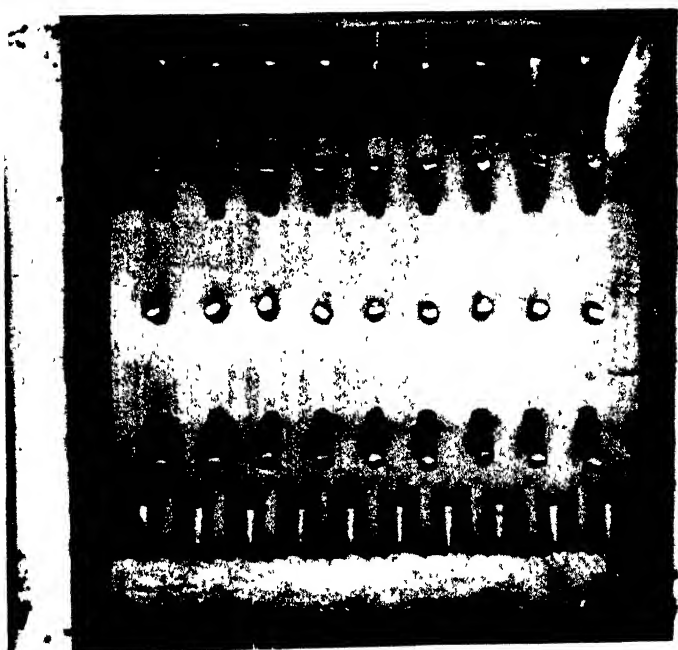
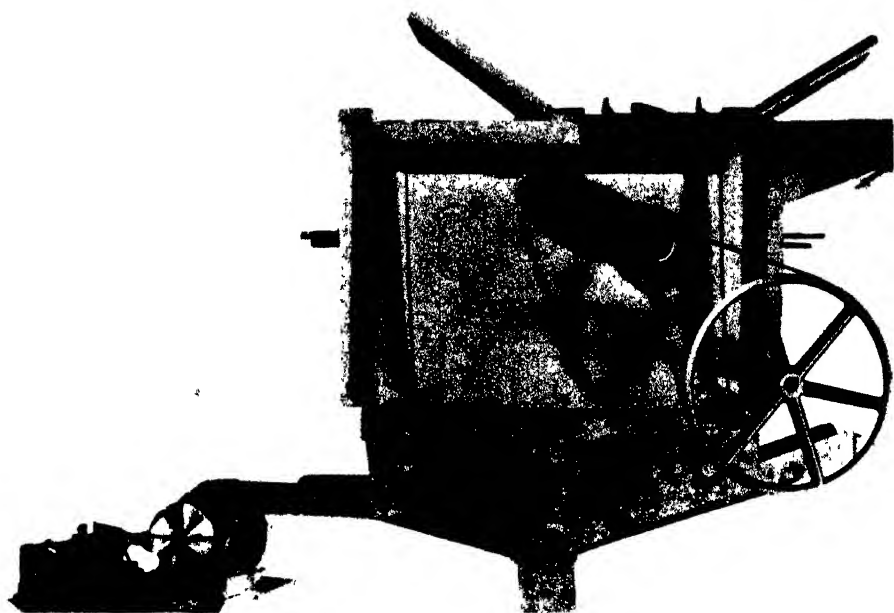


Fig. 1.

between the concave and the drum can be adjusted according to the type of corn which is to be threshed. The space between adjacent teeth is some  $\frac{5}{8}$  in. while the teeth are approximately  $1\frac{1}{4}$  in. long, and  $\frac{1}{4}$  in. in diameter. The mixture of grain, straw, and chaff falls upon the riddle *A* (Fig. 2) of the screening device. The reciprocating motion for this attachment is obtained from a crank situated at the feeding end of the machine, and driven by a belt from the shaft of the drum at about two-sevenths the speed of the latter, the throw of the crank being some 3 in. The grain, chaff, weed seeds, etc., fall through the screen on to the solid part *B*,

<sup>1</sup> Messrs Garvie of Aberdeen supply a "Bench Thresher" whose working part consists of a toothed drum revolving in a toothed concave much smaller than that used at Rothamsted. This was not found satisfactory.







the straw and empty ears are shaken off at *D*, the grain and chaff passing over the edge of this part to meet the air blast entering at *C*. The heavier grain is shaken through the riddle *G*, the lighter empty glumes, awns, etc. are ejected through the opening *E*. The grain is collected in the drawer *F*, which is fitted with a removable screen at the bottom. The mesh of this screen is of such dimensions as just to retain the size of grain it is desired to collect; small corn, weed seeds and other impurities are by this means eliminated.

The power for the thresher was supplied by a 1 H.P. electric motor running at a maximum speed of some 2000 R.P.M. Alteration of the size of the driving pulleys gave suitable speeds for the threshing of different cereals. The blower consisted of a four-bladed rotor driven by an electric

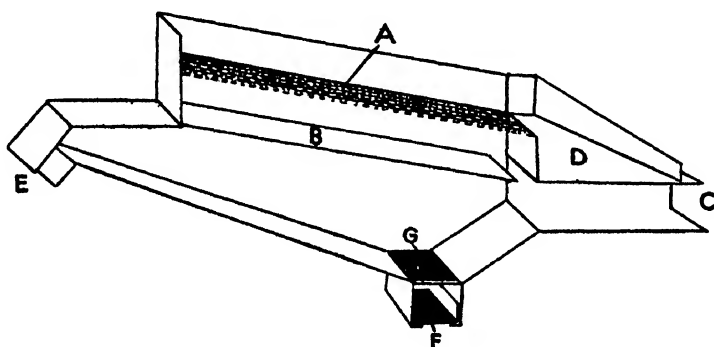


Fig. 2.

motor which was directly coupled to it, revolving at some 2000 R.P.M., giving a calculated output of 220 cu. ft. per minute. The strength of the blast was varied by altering the speed of the motor, a small resistance being used for this purpose.

To attain the maximum speed in dealing with the samples, four operators were needed, and the work was divided among them in the following manner. Having suitably arranged them in a definite order, the first worker weighed the samples and recorded their weights. He then removed the paper bags enveloping the heads of each individual sample, and laid the heads, together with any loose grains upon cardboard trays which he placed ready for the "feeder." The feeding of the machine required the almost undivided attention of another worker. The third was employed in starting and stopping the machine at the beginning and conclusion of each sample, and in removing the full drawers and inserting empty ones. The last worker weighed the dressed grain and recorded its weight. For all weighings Avery A 534 direct-

reading balances were used, their high degree of damping greatly facilitating the speed with which the samples were handled.

With such a disposition of labour it was possible to thresh and finish some 60 separate samples per hour (for one or two short periods a speed of 84 per hour was reached), an achievement which is in itself sufficient testimony to the value of this machine. As mentioned above, barley offered the greatest difficulty, but by the introduction of several minor improvements it is hoped that these difficulties have been overcome.

The upper photograph (Pl. II) shows the machine without driving belt and supports.

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# STUDIES IN SAMPLING TECHNIQUE: CEREAL EXPERIMENTS.

## III. RESULTS AND DISCUSSION.

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(With One Text-figure.)

AFTER threshing there were three sets of figures, representing total weights of sheaflets, weights of grain, and weights of straw. These were subjected to an analysis which aimed firstly at obtaining a direct estimate of the sampling-error, and secondly at comparing the significant results of the experiment, treated as a manurial trial, with those obtained from the "total yields." The analysis was also designed to show whether it was really of value to divide the plot into two or more parts from each of which equal numbers of metre-lengths were cut.

The first step consisted in summing sheaflet yields (whether total, of grain or of straw) and squares of yields, for each half-plot and plot. The plot totals were then compounded in various ways to give sums of sheaflet yields and of squares of sheaflet yields for each block of plots and for each treatment. The grand totals of sheaflet yields and of sums of squares were also calculated.

From these quantities it is no difficult matter to find the total sum of squares of deviations of sheaflet yields from the mean sheaflet yield. This total can be divided into two parts, one representing variations *between* and one *within* half-plots. The former can be further divided into variations *between* different plots, and variations *between* half-plots *within* the same plot. The variation between plots is now divisible into fractions representing variation between blocks of plots; variation between manurial treatments; and lastly, variation due to uncontrolled causes, such as differences in soil fertility between plots in the same block; errors of area measurement, of weighing the manures, etc.; and errors due to sampling. This last fraction affords a basis for estimating the standard error of treatment comparisons.

The labour of calculation was much lessened by the use of a calculating machine.

## 1. ROTHAMSTED WINTER OATS EXPERIMENT.

In this experiment there were sixteen different manurial treatments, and each occurred three times, once in each of three compact "blocks" of plots, the position within the block being assigned at random. The arrangement was therefore an example of Fisher's "Randomised Blocks" method for field experimentation. The sixteen treatments were selected to give as much information as possible about the relative values of sulphate of ammonia and cyanamide, both as spring and autumn dressings. The unit dressing was in all cases equivalent to  $\frac{3}{4}$  cwt. per acre of cyanamide with 19.0 per cent. N. One plot in each block received no dressing; four received single units; six received pairs of different units; four received three different units; and one received all four units. The table shows the manurial scheme.

Table I.

Treatment no.	Treatment	Treatment no.	Treatment
1	—	9	Sa Ca
2	Sa	10	Ss — Cs
3	Ss	11	— Ca Cs
4	— Ca	12	Sa Ss Ca
5	— — Cs	13	Sa Ss — Cs
6	Sa Ss	14	Sa — Ca Cs
7	Sa — Ca	15	— Ss Ca Cs
8	Sa — — Cs	16	Sa Ss Ca Cs

Sa = Sulphate of ammonia applied in autumn.

Ca = Cyanamide applied in autumn.

Ss = Sulphate of ammonia applied in spring.

Cs = Cyanamide applied in spring.

By selection of the appropriate groups of plots it is possible to find the effect of each unit dressing separately, and their "interactions" when two, three or four are present. Every plot can be used for each of these comparisons, so that the arrangement is one of high efficiency.

As has already been stated, the plots, 48 in number, were each  $\frac{1}{40}$ th acre in area, and 30 metre-lengths (15 from each half-plot) were cut from each.

Tables II and III give the complete analyses for grain and straw, both for yields estimated from samples and for "actual yields," obtained by the use of large-scale methods.

The entries in the column headed "Mean square" are obtained from those in the "Sum of squares" column by dividing by the appropriate number of degrees of freedom.

Dealing first with the analysis of "sampling yields," comparison of

Table II.

*Sampling yields (gm. per sampling-unit).*

Fraction	Degrees of freedom	Grain		Straw	
		Sum of squares	Mean square	Sum of squares	Mean square
Blocks	2	1,879.78	939.89	3,838.18	1919.09
Sa	1	8.87	8.87	288.19	288.19
Ss	1	2,014.03	2014.03*	23.46	23.46
Ca	1	1,186.28	1186.28	3,549.46	3549.46
Cs	1	632.03	632.03	2,795.03	2795.03
Sa Ss	1	22.00	22.00	904.40	904.40
Sa Ca	1	238.47	238.47	330.43	330.43
Sa Cs	1	86.53	86.53	94.66	94.66
Ss Ca	1	1,895.21	1895.21*	825.37	825.37
Ss Cs	1	1,713.92	1713.92*	2,188.43	2188.43
Ca Cs	1	22.25	22.25	1,831.06	1831.06
Sa Ss Ca	1	18.45	18.45	158.14	158.14
Sa Ss Cs	1	146.94	146.94	225.47	225.47
Sa Ca Cs	1	35.47	35.47	148.74	148.74
Ss Ca Cs	1	11.74	11.74	767.38	767.38
Sa Ss Ca Cs	1	735.31	735.31	1,102.85	1102.85
Experimental error	30	9,532.16	317.79	28,602.78	953.43
Between half-plots	48	14,268.42	297.26	24,620.84	512.93
Within half-plots	1343	96,328.30	71.73	227,333.66	169.27
Total	1438	130,776.16	—	299,628.52	—

Table III.

*"Actual" yields ( $\frac{1}{4}$  lb. per plot).*

Fraction	Degrees of freedom	Grain		Straw	
		Sum of squares	Mean square	Sum of squares	Mean square
Blocks	2	2,083.04	1041.52	5,755.17	2877.58
Sa	1	75.00	75.00	3,960.33	3960.33*
Ss	1	3,780.75	3780.75*	2,730.08	2730.08*
Ca	1	1,365.33	1365.33*	4,485.33	4485.33*
Cs	1	140.08	140.08	3,780.75	3780.75*
Sa Ss	1	261.33	261.33	4.09	4.09
Sa Ca	1	2.09	2.09	16.34	16.34
Sa Cs	1	8.34	8.34	234.09	234.09
Ss Ca	1	1,976.34	1976.34*	5,084.09	5084.09*
Ss Cs	1	720.75	720.75	5,896.34	5896.34*
Ca Cs	1	1,045.34	1045.34	36.75	36.75
Sa Ss Ca	1	420.08	420.08	200.07	200.07
Sa Ss Cs	1	972.00	972.00	456.32	456.32
Sa Ca Cs	1	352.07	352.07	6.74	6.74
Ss Ca Cs	1	40.33	40.33	47.99	47.99
Sa Ss Ca Cs	1	330.75	330.75	1,160.36	1160.36
Experimental error	30	7,934.30	264.48	15,314.83	510.49
Total	47	21,507.92	—	49,169.67	—



the mean squares corresponding with "between half-plots" and "within half-plots" by means of Fisher's "z" test, shows that both for grain and straw the former is the larger by an amount which would not occur merely by chance as often as once in twenty times. It may be concluded, then, that it has been advantageous to divide the plots transversely, and to take half the total number of metre-lengths from each half-plot. For had this not been done, there would have been plots on which the metre-lengths came nearly all from one half; and the accuracy of the yield estimate would have been diminished, since the two halves are shown to differ significantly.

The fraction "within half-plots" represents the variation between metre-lengths of the same half-plot, and provides a direct estimate of the sampling-error, since all variation due to treatment and position of the plot, and to differences between half-plots of the same plot, are here eliminated. The square root of the mean square gives the sampling-error of a single metre-length. Since there are 30 metre-lengths from each plot, the sampling-error of a plot mean is obtained from this by dividing by  $\sqrt{30}$ .

By treating the "experimental error" mean square in exactly the same way, an estimate is obtained of the variability of the "sampling yield" of a single plot when correction has been made for the average fertility of the block in which it falls, and for the manurial treatment which it has received. It thus includes errors due to sampling as well as those due to differences in fertility between plots of the same block, and to working errors; and is the appropriate basis for determining the significance of manurial effects.

In the case of "actual yields" only one of these quantities can be calculated—the experimental error per plot—and this is simply the square root of the corresponding mean square.

Table IV gives the values of these errors for grain and straw, and for both sets of data.

Table IV.

	Grain			Straw		
	Mean square	Standard error	per cent. per plot	Mean square	Standard error	per cent. per plot
(a) "Sampling yields":						
Experimental error	317.74	3.254	12.36	953.43	5.638	13.09
Within half-plots	71.73	1.546	5.87	169.27	2.375	5.51
(b) "Actual yields":						
Experimental error	264.48	16.26	11.07	510.49	22.59	7.90

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The sampling-errors are higher than the expected 5 per cent. per plot. This is almost certainly due to winter mortality, many plants being killed by the severe frosts of February, 1928. Despite this the experimental error per plot has been only slightly increased in the case of grain (11.07 to 12.36 per cent.). The increase is more serious for straw (7.90 to 13.09 per cent.); reasons for this will be discussed later.

The real test of the adequacy of a sampling method is the comparison of the amount of information obtained by its use, with that obtained when large-scale methods are employed. Tables II and III give complete analyses of the effect of the various manurial treatments. The test of significance consists in comparing, by means of the "z" distribution, the appropriate mean square with the "experimental error" mean square. Those items which show a significantly higher variation than that due to experimental error (taking odds of 1 in 20 as the level of significance) are marked with an asterisk. It will be seen that, for grain, both sets of data yield three significant items, of which two are common, Ss, and the first order interaction SsCa. The autumn application of cyanamide (Ca), which would be judged effective on the basis of the analysis of "actual yields," just fails to reach the 1 in 20 level in the analysis of "sampling yields"; and the first order interaction of the two spring dressings (SsCs) is significant in the latter but not in the former analysis. Substantially the same results are thus obtained by the two methods: the differences may be due to differences in the mesh of the dressing screens.

For straw the differences are much more striking. While no less than six items are starred in Table III, there are none in Table II (Ca just fails to reach the 1 in 20 level). This is the more curious in that the sampling-error per plot is actually lower for straw (5.51 per cent.) than for grain (5.87 per cent.). Two factors seem to be at work here. In the first place the height above ground at which the straw was cut was constant for the large-scale method, where a binder was used; but varied a little from plot to plot where several different workers were cutting samples. Secondly, the crop was very weedy, owing to the thinness of the plant after the winter frosts, and since all the weeds were included in the "large-scale" sheaves, but were partially discarded from the sampling sheaflet, the yields of straw would be expected to differ on this account. The effect of the first factor would be to increase the experimental error as calculated from the sampling data. This would tend to obscure real effects of manurial treatment. A hint that this surmise is correct is obtained by comparing the difference between the two

estimates of experimental error with the sampling-error. If the only important additional source of variation is the sampling technique, then

$$V_s = V_1 - V_2,$$

where  $V_s$  = relative variance due to sampling,

$V_1$  = relative variance corresponding with experimental error for "sampling" yields,

$V_2$  = relative variance corresponding with experimental error for "actual" yields.

For the relative variances we may use the squares of the sampling and experimental percentage errors. Then, for grain:

$$V_s = 5.87^2 = 34.46; V_1 - V_2 = 12.36^2 - 11.07^2 = 30.23$$

and the agreement is good.

For straw, however:

$$V_s = 5.51^2 = 30.36; V_1 - V_2 = 13.09^2 - 7.90^2 = 108.94.$$

There is here a considerable difference in the expected direction, supporting the view that the experimental error calculated from the sampling data differs from that calculated from the "actual" yields by another important factor in addition to the sampling-error, this being the variation in the length of straw cut from the different plots.

Support for the second assumption is found in comparing the estimates of average yield and the ratios of straw to grain obtained by the two methods (Tables V and VI).

Table V. *Mean yield in cwt. per acre.*

Grain	"Actual yields"	...	...	13.12
	"Sampling yields"	...	...	13.77
Straw	"Actual yields"	...	...	25.54
	"Sampling yields"	...	...	22.52

Table VI. *Ratio of straw to grain.*

"Actual yields"	...	...	...	1.95
"Sampling yields"	...	...	...	1.64

Here the estimates of mean yield of straw differ by more than 12 per cent. in favour of the large-scale method, as would be expected if a considerable quantity of weed were weighed with the sheaves. Further evidence for this view is that the discrepancies between the estimates of straw yield are greater for plots receiving sulphate of ammonia than

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for plots receiving cyanamide, a result to be expected if cyanamide depresses the germination of weed seeds.

This is an important conclusion, since the significant effects which emerge from analysis of the "actual yields" are now under suspicion as being probably due in part to differences in amount of weed infestation. The sampling method, it should be noted, provides a means of estimating this disturbing factor on experimental plots, or, alternatively, of eliminating it.

### 2. ROTHAMSTED BARLEY EXPERIMENT.

The arrangement of this experiment has already been described (p. 367). The important feature from the point of view of the sampling method was that each of the 50 plots was divided into quarters, 1/160th acre in area, which received different manurial treatments. The separate harvesting of these quarter-plots was effected only by the sampling method; the task would have been very difficult or impossible if large-scale machinery had to be employed.

The sampling and experimental errors are shown in Table VII.

Table VII.

	Grain		Straw	
	Whole plot	Quarter-plot	Whole plot	Quarter-plot
A. Square at the lower level of nitrogen.				
(a) "Sampling yields":				
Experimental error	5.58	13.70	13.56	14.99
Sampling-error	5.53	11.07	5.58	11.17
(b) "Actual yields":				
Experimental error	8.46	—	10.04	—
B. Square at the higher level of nitrogen.				
(a) "Sampling yields":				
Experimental error	6.98	10.64	7.58	11.71
Sampling-error	4.99	9.97	5.09	10.19
(b) "Actual yields":				
Experimental error	3.77	—	6.26	—

The sampling-errors for whole plots are those of means of 32 sampling-units, and are comparable with the values obtained in the winter oats experiment, 5.87 per cent. for grain and 5.51 per cent. for straw. It will be seen that the expected value of about 5 per cent. was obtained in plots which received the double quantity of nitrogen. It is almost invariably found, as here, that an area bearing a heavy crop gives smaller experimental errors than one otherwise similar but with a light crop. The fact that the sampling-errors are also smaller suggests that the ex-

planation lies in the greater capacity of a heavily manured crop to compensate for unevenness in the original plant. In a cereal crop this compensation usually takes the form of an increased number of ear-bearing tillers on the plants adjoining gaps.

The very small experimental error for "actual yields," especially of grain, in plots receiving the heavy dressing, exaggerates the difference between the two methods of harvesting. An error as low as 3.77 per cent. must be regarded as exceptional, however; usually the plot error falls between 6 and 12 per cent. of the mean yield.

Only eight sampling-units were taken from each quarter-plot, and the sampling-errors are double those for whole plots.

Table VIII. "*Sampling yields*" (gm. per quarter-plot).

Fraction	Degrees of freedom	Grain		Straw	
		Sum of squares	Mean square	Sum of squares	Mean square
A. Square at lower level of nitrogen.					
Rows	4	9,787.69	2,446.92	19,456.86	4,864.22
Columns	4	55,412.72	13,853.18*	55,328.13	13,832.03
Treatments	4	45,993.74	11,498.44*	66,126.06	16,531.52
Error ( <i>a</i> )	12	18,146.00	1,534.67	112,909.43	9,409.12
P	1	0.81	0.81	1.32	1.32
K	1	306.25	306.25	95.06	95.06
PK	1	169.00	169.00	1,447.80	1,447.80
Nit. × P	1	122.10	122.10	666.93	666.93
Nit. × K	1	473.06	473.06	405.02	405.02
Nit. × PK	1	5,076.56	5,076.56	7,881.00	7,881.00
Qual. × P	3	10,417.34	3,472.45	9,069.98	3,023.33
Qual. × K	3	13,382.64	4,460.88	15,520.21	5,173.40
Qual. × PK	3	11,042.24	3,680.75	16,495.53	5,498.51
Error ( <i>b</i> )	60	138,730.75	2,312.18	172,364.60	2,872.74
Total	99	355,054.64	—	477,767.93	—
B. Square at higher level of nitrogen.					
Rows	4	43,989.12	10,997.28*	121,172.74	30,293.18*
Columns	4	5,816.61	1,454.15	27,418.91	6,854.73
Treatments	4	27,489.22	6,872.31	77,191.89	19,297.97*
Error ( <i>a</i> )	12	38,643.32	3,220.28	46,642.55	3,886.88
P	1	53.29	53.29	64.00	64.00
K	1	14,328.09	14,328.09*	15,951.69	15,951.69*
PK	1	11.66	11.66	234.09	234.09
Qual. × P	4	9,405.79	2,351.45	14,122.78	3,530.70
Qual. × K	4	9,036.79	2,259.20	18,242.49	4,560.62
Qual. × PK	4	4,550.92	1,137.73	5,434.24	1,358.56
Error ( <i>b</i> )	60	112,254.93	1,870.92	139,255.08	2,320.92
Total	99	265,579.64	—	465,730.46	—

Error (a) is the basis for direct comparison of whole-plot treatments, and error (b) for quarter-plot treatments and their interactions with the whole-plot (nitrogenous) treatments. Nit. × P, etc. are interactions of the quarter-plot treatments with nitrogen, irrespective of the form in which the nitrogen is applied. Qual. × P, etc. are differential responses to the quarter-plot treatments on plots receiving nitrogen in different forms.

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The analyses of variance for "sampling yields" and "actual yields" are given in Tables VIII and IX, fractions significantly exceeding that ascribable to experimental error being marked with an asterisk.

Table IX. *Actual yields ( $\frac{1}{4}$  lb. per plot).*

Fraction	Degrees of freedom	Grain		Straw	
		Sum of squares	Mean square	Sum of squares	Mean square
A. Square at lower level of nitrogen.					
Rows	4	2,205.04	551.26	1,533.04	383.26
Columns	4	2,161.84	540.46	8,194.24	2048.56*
Treatments	4	10,175.44	2543.86*	13,073.84	3268.46*
Error	12	5,808.52	484.04	8,387.92	698.99
Total	24	20,351.84	—	31,189.04	—
B. Square at higher level of nitrogen.					
Rows	4	5,519.04	1379.76*	7,429.60	1857.40*
Columns	4	6,080.24	1520.06*	3,246.80	811.70
Treatments	4	2,668.24	667.06*	3,865.20	966.30*
Error	12	1,463.92	121.99	3,838.40	319.87
Total	24	15,731.44	—	18,380.00	—

It will be seen that whole-plot treatments appear effective in all cases when "actual yields" are analysed, but that "sampling yields" fail to show an effect on straw at the lower level and on grain at the higher level of nitrogen. These results are shown in the table of percentage yields (Table X).

Table X.

A. At lower level of nitrogen.						Standard error	Mean
Grain	O	S	M	N	C	Mean	(cwt. p.a.)
Actual	86.4	99.6	101.6	110.5	101.9	100.0	3.79
Sampling	90.2	103.1	102.7	107.7	96.3	100.0	22.21
Straw						2.50	22.93
Actual	86.4	101.6	98.3	113.7	100.0	100.0	4.49
Sampling	88.4	101.6	103.2	110.1	96.7	100.0	23.51
Straw						6.06	23.37
B. At higher level of nitrogen.						Standard error	Mean
Grain	U	S	M	N	C	Mean	(cwt. p.a.)
Actual	97.0	96.3	100.0	106.3	100.4	100.0	1.68
Sampling	93.7	98.0	99.5	105.1	103.6	100.0	26.19
Straw						3.12	26.57
Actual	94.3	97.7	100.0	107.5	100.6	100.0	2.80
Sampling	94.0	95.1	94.6	106.3	110.0	100.0	25.50
Straw						3.39	26.89

O=no nitrogen; S=sulphate of ammonia; M=muriate of ammonia; C=cyanamide; N=nitrate of soda; U=urea.

The information lost is in each case the significant superiority of nitrate of soda to other sources of nitrogen, found in all cases for "actual

yields," but only for grain at the lower level for "sampling yields." At the higher level of nitrogen the "sampling yields" of straw show both cyanamide and nitrate of soda significantly above the other three forms of nitrogen. This difference in the position of cyanamide is curious, but is perhaps due to the partial removal of weeds from sampling sheaflets, as in the oats experiment. It is frequently claimed for cyanamide that it inhibits the germination of weed seeds: if there were a real lessening of the weight of weeds on plots treated with this fertiliser, the effect would be that observed.

The sampling method is shown to better advantage in the quarter-plot results. It will be remembered that the quarter-plots were only 1/160th acre in area, and could hardly have been dealt with by large-scale methods. The quarter-plot treatments were identical for each whole plot: (1) no additional treatment; (2) superphosphate; (3) sulphate of potash; (4) both superphosphate and sulphate of potash: the allocation of the four treatments to the quarter-plots within any plot was at random. The analyses of Table IX show that neither phosphate nor potash was effective at the lower level of nitrogen, but that at the higher level there was a significant response to potash both in grain and straw. As Table XI shows, this response was a depression in yield. Its magnitude was quite small—5·88 per cent. for grain and 6·14 per cent. for straw—but the low standard error makes even so small a difference significant. This is a striking demonstration of the efficiency of the experimental arrangement, as well as an example of the manner in which sampling can act as a valuable auxiliary to large-scale harvesting methods.

No differential responses to potash or phosphate on plots bearing different forms of nitrogen were detected, as is shown in the analyses.

Table XI.

	O	P	K	PK	Mean	Standard error
A. At lower level of nitrogen (percentage of mean yield).						
Grain	100·84	100·15	99·11	99·90	100·00	2·74
Straw	100·82	98·63	99·24	101·30	100·00	3·00
B. At higher level of nitrogen.						
Grain	102·85	103·04	96·79	97·32	100·00	2·13
	102·94		97·06			
Straw	102·50	103·64	97·11	96·75	100·00	2·34
	103·07		96·93			

## 3. ROTHAMSTED WHEAT EXPERIMENT.

The wheat experiment of 1928-9 was designed to give information as to the effect of applying sulphate and muriate of ammonia as top dressings to four different varieties of wheat. The top dressings were given either early (March 18), late (May 13), or at both these dates. Owing to the severe frosts of February and March 1929 the plant was very thin, and later the plots became infested with Black Bent (*Alopecurus agrostis*). The weediness was much more marked at one side of the experimental area than at the other, and tended to increase still further what must in any case have been a large experimental error. As a result no treatment or variety differences could be regarded as significant, and no more information was obtained by the large-scale than by the sampling method. Table XII gives the sampling and experimental errors per plot, expressed as percentages of the mean yield.

Twenty-four metre-lengths of drill were cut from each of the plots. The area of each plot was 1/55th acre.

Table XII.

	Grain		Straw	
	(a)	(b)	(a)	(b)
(a) "Sampling yields":				
Experimental error	11.69	20.39	14.41	16.89
Sampling-error		5.84		6.73
(b) "Actual yields":				
Experimental error	9.68	22.48	9.99	15.14

Columns (a) and (b) show the plot errors for varietal and treatment comparisons respectively.

## 4. WELLINGORE BARLEY EXPERIMENT.

By courtesy of G. H. Nevile, Esq., of Wellingore Hall, Lincs., an experiment was carried out on Lincoln Heath, near the village of Wellingore. This consisted of sixteen plots, bearing eight different treatments in duplicate—no artificial fertilisers, and any one, two, or three of the following dressings: sulphate of ammonia at 1 cwt. per acre, sulphate of potash at 1 cwt. per acre, and superphosphate at 3 cwt. per acre. The plots were each 1/60th acre, and were harvested only by a sampling method. Forty half-metre lengths of drill were cut from each plot, but actually there were only four sampling units—*i.e.* four independently located parts of the sample (2). The procedure was to select four drill-rows at random from each plot (discarding edge-rows), and



then to cut 10 half-metre-lengths as shown in the diagram (Fig. 1). The measuring-rod was that used at Rothamsted, 2 half-metres being separated by a metre.

A constant number of paces separated successive placings of the rod. It is readily seen that this method gives a complex sampling-unit which involves four rows, and that four such sampling-units must provide a satisfactorily representative sample of the produce of the plot.

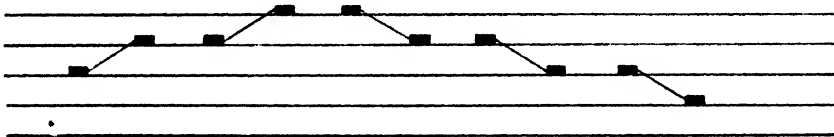


Fig. 1.

The analyses of variance for grain and straw are shown in Table XIII, fractions significantly exceeding that ascribable to experimental error being marked with an asterisk.

Table XIII.

Fraction	Degrees of freedom	Grain		Straw	
		Sum of squares	Mean square	Sum of squares	Mean square
Blocks	1	6,172.07	6,172.07	744.27	744.27
K	1	2,217.82	2,217.82	2,960.04	2,960.04*
P	1	962.94	962.94	1,567.67	1,567.67*
N	1	17,797.23	17,797.23*	29,745.47	29,745.47*
PK	1	199.52	199.52	345.73	345.73
NK	1	826.56	826.56	2,629.77	2,629.77*
NP	1	14,475.10	14,475.10*	11,469.07	11,469.07*
NPK	1	2,044.73	2,044.73	2,604.18	2,604.18*
Error	7	4,551.94	650.28	2,021.19	288.74
Within plots	48	22,936.90	477.85	22,740.95	473.77
Total	63	72,184.81	—	76,828.34	—

The sampling-error per plot was 5.31 per cent. for grain, and 5.78 per cent. for straw; and the experimental errors 6.19 per cent. and 4.51 per cent. respectively.

It is interesting to note that for grain two, and for straw no less than six of the treatment items were found to be significant. The low experimental errors which make the experiment so useful can doubtless be ascribed to the exceptional uniformity of the soil and the plant. That the plant was uniform is further shown in the magnitude of the sampling-errors, which are of the same order as in Rothamsted experiments where 30 or more metre-lengths were taken from each plot.

## DISCUSSION.

The results are summarised in Table XIV. The experiments with barley certainly justify the claims made in an earlier paper for the accuracy and usefulness of the sampling method described. The sampling-error per plot has been rather more than 5 per cent. of the mean yield; the experimental error, as for grain at the lower level of nitrogen in the barley experiment, may actually be lower than the corresponding large-scale figure; little information has been lost which a large-scale method would have given; plots were successfully dealt with which would have been much too small for large-scale experimentation; and the large-scale methods were entirely dispensed with in an outside experiment which yielded a great deal of information as to the effects of various fertiliser combinations. Further advantages are that edge-rows can be discarded without the necessity of removing them; losses in the stook and in the stack are avoided; results are available sooner than would normally be the case with stacked corn; and the bulked produce of the independently located sampling-units constitutes an excellent sample for analytical work.

Table XIV.

Crop	Size of sample (metres)	Area of plot (acre)	Sampling-error (% per plot)	Experimental error (% per plot)	
				(a) "Sampling yields"	(b) "Actual yields"
Wheat:					
1. Grain	24	1/55th	5.84	11.69, 20.39	9.68, 22.48
2. Straw			6.73	14.41, 16.89	9.99, 15.14
Oats:					
1. Grain	30	1/40th	5.87	12.36	11.07
2. Straw			5.51	13.11	7.90
Barley:					
(a) 1. Grain	32	1/40th	5.53	5.58	8.46
2. Straw			5.58	13.56	10.04
(b) 1. Grain	32	1/40th	4.99	6.98	3.77
2. Straw			5.09	7.58	6.26
Barley (Wellingore):					
1. Grain	20	1/60th	5.31	6.23	—
2. Straw			5.78	4.51	—

The experiments with winter-sown cereals were somewhat less pleasing, but the higher sampling-error per plot can almost certainly be ascribed to the depletion of plant by the severe frosts of the winter 1928-9. Little useful information was derived from the wheat experiment. From the results of the oats experiment, however, it is shown that the sampling method can be used to give the weight of straw freed from weeds, and also an estimate of the effect of various fertilisers on weed growth.

Where large-scale equipment is already in use it could hardly be suggested that this should be entirely replaced by the apparatus necessary for the sampling method. The results of the 1929 experiments show, however, that sampling for yield might well be adopted as an auxiliary method, and where no large-scale machinery is already available it would further recommend itself through the relative cheapness of the necessary equipment. It solves the problem of harvesting complex experiments on farms at some distance from the organising station, and by thus permitting the repetition of experiments on many types of soil, greatly enhances their value.

The practicability of dealing with small plots is an important point. It has been shown by Roemer(4) and others that for a given experimental area, to be used for the comparison of a given number of varieties or treatments, it is of much greater advantage to increase the number of replications than to increase the size of the individual plot. In other words, the loss of accuracy arising from reduction in the size of the individual plot is more than counterbalanced by the gain from a higher degree of replication. The labour of sampling, however, from the experimental area may not be greatly increased by an increase in the number of plots into which it is divided, and the absolute size of the individual plot does not in any way affect the practicability of sampling. The extent to which the total size of sample taken from the area is altered depends, of course, on the nature of the variations in yield per unit length of drill over the area. Thus if the mean yield of a small plot (for constant treatment), and the variability within the plot, were fairly constant throughout, it would be necessary to take almost as many sampling-units from a small plot as from a large plot. If, on the other hand, mean fertility varied considerably between small plots, it would be possible to reduce the number of sampling-units when the plot-size is reduced. It may be said in general that the number of sampling-units to be taken from a small plot can be at least  $(n - 1)$  less than the number taken from a larger plot, where the areas are in the ratio  $1:n$ , provided that it proved profitable to subdivide the larger plot into  $n$  parts for the purpose of sampling. The test of the advantage gained by subdivision is the significance of the difference between the mean squares for "within subdivisions" and "between subdivisions," as explained on p. 379.

It may be noted that it is not essential to take a large number of sampling-units from each plot, though in exploratory work such as that described it was desirable in order to obtain an accurate estimate of the sampling-error, of the advantage gained by subdivision of plots, etc. When such preliminary work has been completed, it should be sufficient

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to take two or three complex sampling-units from each plot, the size of the sample remaining, of course, unchanged.

In conclusion it should be pointed out that for convenience in sampling the distance between drills should be not less than 7 in., and that where choice is possible, a stiff-strawed variety should be grown, since lodged corn is very difficult to sample adequately.

### SUMMARY.

1. Four cereal experiments, comprising 210 plots each about 1/40th acre in area, were harvested by a sampling method. Three of the experiments were later harvested by large-scale methods, so that a direct comparison could be made.

2. The field technique is described, and an account is given of the small combined thresher and winnower which was constructed for the purpose of dealing rapidly with the numerous small sheaflets.

3. The results are analysed in detail and it is shown that the sampling-errors per plot lie between 5 and 6 per cent. of the mean yield, and that these errors are sufficiently low for there to be little loss of information.

4. The relative advantages of large-scale and sampling methods are discussed, with special reference to the possibility of dealing with large numbers of very small plots, and of carrying out complex experiments on farms distant from the organising station.

Finally, it is with pleasure that we record our indebtedness to Messrs Garner, Parbery, Hansen, Leonard, French, Weston, Cole and others for assistance in the field and with the threshing; to Dr J. Wishart for providing the analyses of "actual yields," and to Dr R. A. Fisher for constant readiness to offer suggestions which were always valuable.

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# Field Observations on Starch Production in the Leaves of the Potato.

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With two Figures in the Text.

THE effect upon yield of crop plants of such changes in their environment as can be brought about by cultivation or manuring offers many problems to the plant physiologist. Among the most interesting of these problems is that of the analysis of the observed effect upon yield in terms of the effect upon the various processes at work in the plant. For the purpose of this inquiry, it is a great advantage if the field experiment can be arranged so as to furnish not only data for yield but also data concerning those aspects of plant behaviour in terms of which the analysis of yield may be attempted. Manurial experiments offer very favourable material for studies of this type, and the present paper is concerned with some preliminary work on one selected aspect of the behaviour of potatoes in a manurial experiment which has been carried out for the past four years at Rothamsted by Mr. T. Eden.

The work has covered so far only the later period of growth of the plants, and a discussion of the results in relation to quantity and quality of yield is not as yet possible. The chief interest of the present paper lies rather in the statistical analysis of the experimental results and in the evidence which the results supply of the usefulness for field studies of the method employed. The bearing of the work upon the problems of yield will, however, become clearer if some account is first given of the nature of the potato experiment and of the yield results which have so far been obtained. For this summary the writer is indebted to Mr. T. Eden.<sup>1</sup>

The experiment is a comparison of the effect upon the yield of potatoes of potash, supplied in three different forms. The four manurial treatments involved are : (1) Control, i. e. no potash ; (2) potash as potassium sulphate

<sup>1</sup> See also Journ. Ministry of Agriculture, Jan., 1926. Report of Potato Conference.

(47.71 per cent.  $K_2O$ ); (3) potash as potassium chloride (52.11 per cent.  $K_2O$ ); (4) potash as 'potash manure salts' (P.M.S.) (27.65 per cent.  $K_2O$ ). The quantities of the three potash manures are so adjusted that all plots receive an amount of potassium per acre equal to that in 2 cwt. of potassium sulphate. The basal manuring for all plots is 2 cwt. per acre of sulphate of ammonia and 6 cwt. per acre of superphosphate. The variety of potato employed is Kerr's Pink (Scotch seed).

In considering the yield results so far obtained we may distinguish two main questions: (1) the effect of 'potash' as against 'no potash'; (2) the effect, within the group of potash manures, of the other substances associated with the potash. The results show a significant superiority of 'potash' over 'no potash' in yield of tubers, the magnitude of the potash effect being a function of season. As between the three forms of potash there is no significant difference that is consistent from year to year, but there seems to be a significant differential response to season, the P.M.S. plots giving relatively low yields in some years. The starch content of the tubers is, however, not the same for all the potash manures. The values vary with manurial treatment as well as with season, the averages for three years being  $K_2SO_4$  62.17 per cent., KCl 59.72 per cent., P.M.S. 57.13 per cent., 'no potash' 58.07 per cent. When this effect upon starch content is considered in relation to yield the differences between the various treatments become significant, the mean value of starch per acre for the three years being  $K_2SO_4$  1.3243, KCl 1.2180, P.M.S. 0.9643, 'no potash' 0.8377.

It will be seen that the relation of these manurial treatments to the production of starch by the potato plant is of considerable interest, and a study of the behaviour of the leaves as regards production and removal of starch seemed desirable.

For the purpose of this field study on the experimental plots some simple method which would allow of simultaneous observations on all four plots was required, and Sachs's 'iodine' method seemed likely to be useful. This method has been used as a quantitative one by Ursprung<sup>1</sup> in a study of the influence of light of different wave-lengths upon starch formation, and the method which he employed, of grading the amount of starch formed by comparing the colour developed in the iodine test with a standard scale of colour tones, has been adopted here. Preliminary trials showed that matching of the tones was easier and more accurate if a scale containing some violet colour was used rather than a scale of neutral or carbon grey. The scale finally selected was No. 59<sup>100</sup> in Ridgway's 'Colour Standards and Nomenclature' (Washington, D.C., 1912). The scale in question consists of nine tones; a central colour, No. 5 in the scale, being modified by progressive increments of white (tones 4, 3, 2, 1) or of black (tones 6, 7, 8, 9); the

<sup>1</sup> Ursprung, A.: Ber. d. Deutsch. Bot. Ges., Bd. xxxv, p. 44, 1917.

increments of white being 9.5 per cent., 22.5 per cent., 45 per cent., and 100 per cent., and of black being 45 per cent., 70.5 per cent., 87.5 per cent., and 100 per cent. The central tone is a broken colour containing 95.5 per cent. of neutral grey and 4.5 per cent. of violet (wave-length 410). The neutral grey is equivalent to a colour-wheel mixture of black 79 per cent., white 21 per cent. The colour-wheel analysis of the scale from Ridgway's data is shown in Table I.

TABLE I.

*Analysis of Colour Tone Scale, Ridgway 59<sup>1111</sup>.*

<i>Tone No.</i>	<i>White %.</i>	<i>Black %.</i>	<i>Violet %.</i>
1.	100	—	—
2.	55.77	41.75	2.48
3.	37.67	58.84	3.49
4.	27.22	68.71	4.07
5.	19.58	75.92	4.50
6.	10.77	86.75	2.48
7.	5.78	92.89	1.33
8.	2.45	96.99	0.56
9.	—	100	—

The colour intensity developed by the iodine test in any leaflet may therefore be expressed as a number on the scale of tones, or as a percentage of black; and, similarly, differences in colour intensity between different leaflets may also be expressed in either units. It will be seen, however, that successive increments on the tone scale from 1 to 9 correspond to progressively smaller increments of percentage of black. Thus in estimating starch production by differences in colour intensity shown by the iodine test much depends on the scale in which the results of the colour grading are expressed. In the absence of precise information as to the relation between starch content and the colour tone shown by the iodine test (information which it is hoped to obtain next year), the results presented in this paper have been calculated on both bases, greater weight being assigned to the tone scale basis as representing the primary data. There is, moreover, some evidence which suggests that the tone scale does roughly correspond with starch content over the greater part of its range.

This evidence is derived from tests which were made with a series of solutions of soluble starch in distilled water, ranging in concentration from 0.0065 per cent. to 1.664 per cent. One c.c. of each solution was run evenly on to a No. 1 Whatman filter-paper of 9 cm. diameter; two filter-papers being prepared in this way from each solution. The eighteen filter-papers were then dried in an oven at 90° C. for three hours. Four squares of about 2 cm. diameter were cut from each, the whole set stained in iodine solution as in Sachs's test, washed in running water till no trace of brown discoloration due to iodine remained on the filter-paper, spread on

the surface of a white plate, and compared with Ridgway's colour scale 59<sup>IIII</sup>.

The average colour value for the eight squares prepared from each solution is given in Table II.

TABLE II.

<i>Starch Solution.</i>	<i>Mean Colour Tone.</i>
%.	
0.0065	< 1.25
0.0130	< 1.25
0.0260	1.35
0.0520	1.65
0.1040	2.25
0.2080	3.20
0.4160	4.65
0.8320	7.10
1.6640	8.50

The increment of starch content corresponding to a single step in the tone scale increases as we go up the tone scale: this increase is relatively slow between tones 1 to 7, but very rapid after that. In the experiments on the potato leaves the colour intensity observed rarely exceeded tone No. 7, and, in view of the relatively small departure from a linear relation between tone scale and starch concentration over the range 1-7, that scale may be considered the best simple approximation for the estimate of changes in starch content in the experimental leaflets.<sup>1</sup>

#### *Experimental Procedure.*

The object in view was to obtain, at different periods during growth, estimates of the net amount of starch produced, during a specified time interval, by leaves of plants on the experimental plots. This net starch production was estimated as the difference between the colour value developed in the iodine test by a leaflet exposed to light on the plant for three hours and the colour value of *its opposite leaflet* which had remained covered during the three hours. On the assumption that loss of starch due to translocation and respiration proceeds as rapidly in the covered leaflet as in the exposed, the difference between the two should be a measure of net starch production and would correspond to an estimate of real assimilation. In as far as the rate of loss of starch by translocation, or of carbohydrate which might other-

<sup>1</sup> It may be of interest to note the amounts of starch required on the basis of the above figures to give the colour tones observed in the case of the potato leaflets. These are, for the potassium sulphate plot :

Mean colour tone,	{ covered leaf 1.78 $\equiv$ 0.511 mg. starch per 50 sq. cm. filter-paper.
	{ exposed leaf 4.5 $\equiv$ 3.270 " " " "
Difference (= starch production)	$\equiv$ 2.759 " " " "



wise have become starch, is a function of carbohydrate concentration in the leaflet, this rate of loss should be somewhat greater in the exposed leaf, and the estimate of net starch production will therefore be a minimum one.

The procedure in making the observations was as follows: One plot of each treatment was chosen from the field experiment, which was in the form of a Latin square,  $4 \times 4$ , each plot  $\frac{1}{80}$  acre in area. The four plots chosen formed one row of the experiment and had been arranged at random within the row. Between 5 and 6 p.m. on the evening before observations were to be made a pair of opposite leaflets on each of twelve plants on each of the four plots were covered by black paper envelopes, these being secured, without damage to the petiole, by paper clips partially closing the opening by which the envelope was slipped on to the leaflet.

At about 9.30 a.m. on the following day the black envelope was removed from one leaflet of each of six plants on each plot. Three hours later the six leaflets thus exposed were, together with their opposite (covered) leaflets, removed from the plant and taken to the field laboratory. At about the same time envelopes were removed from one leaflet of each of the six remaining marked plants on each plot, and again three hours later both the exposed and the covered leaflets were removed and taken to the field laboratory. On being brought into the field laboratory the leaflets were at once boiled for ten minutes in water, decolorized in warm alcohol, stained in a solution of iodine in potassium iodide, and then washed in tap-water until no traces of free iodine remained. They were then spread on a white enamel plate and their colour compared with the Ridgway scale. Up to this point each of the samples of six leaflets was treated as a unit, as it was not found practicable to keep the exposed and covered leaflets in their original pairs. The colour tone of each leaflet was estimated separately.

The leaflets used were in all cases a pair of opposite leaflets next the terminal leaflet on the fourth leaf from the apex of a haulm, since a preliminary survey had shown that these were usually of a convenient size and had a fairly uniform distribution of starch. Older leaflets were, especially on the potash-starved plot, liable to marked patchiness in starch distribution. Throughout, only leaves which were green and apparently healthy were used.

### *Experimental Results.*

Owing to the pressure of other work it was not possible to begin the observations until September. The plots had been planted on April 30, and the plants had appeared above ground about June 15. By the beginning of September growth of the haulms had almost ceased, and the observations relate therefore to the final stages of the vegetative life-cycle. The appearance of the plants at this time may be summarized as follows: (a) Potassium sulphate plot. Plants of a bushy habit, foliage a healthy green, but the

older leaves beginning to yellow and ripen off during the month. (b) Potassium chloride plot. Plants somewhat taller but less bushy than on the sulphate plot: foliage green, but the older leaves yellowing earlier than on the sulphate plot. (c) P.M.S. plot (30 per cent. potash manure salts). Plants similar to those on the potassium chloride plot, but the older leaves yellowing somewhat earlier. (d) No potash plot. Plants stunted and most of

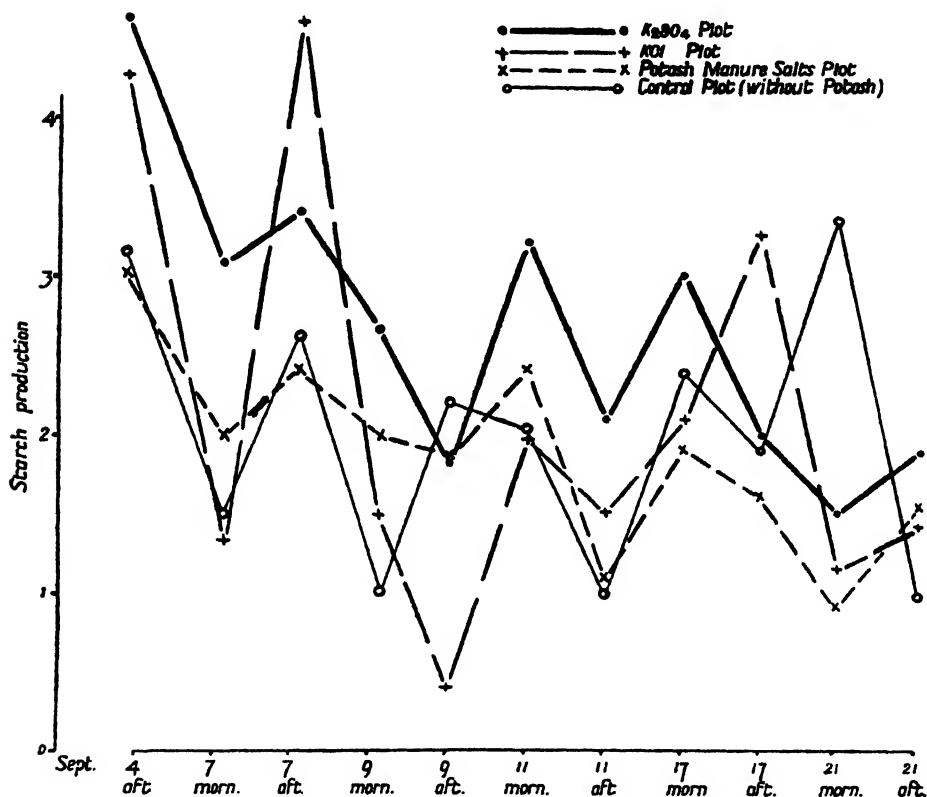


FIG. 1.

the older leaves discoloured with copper-coloured patches (often areas of local death).

The results of all the experiments in which complete data from all four plots were secured are given in Table III, and the values for starch production are shown in Fig. 1. The figures in the table are the means of the tone values for the six leaflets of each sample. The values for starch production are the differences between the values for the exposed and covered leaflets and refer to a period of three hours. (The first experiment lasted three hours and twenty minutes, and the figures for starch production have been corrected for three hours.) The mean values for each occasion irrespective

TABLE III.  
Starch Production in Potato Leaflets (Tone Scale Values).

Treatment →	K <sub>2</sub> SO <sub>4</sub>			No Potash.			P.M.S.			KCl.			Mean.		
	Exposed.	Covered.	Starch Production.	Exposed.	Covered.	Starch Production.	Exposed.	Covered.	Starch Production.	Exposed.	Covered.	Starch Production.	Exposed.	Covered.	Starch Production.
Date. Period.															
Sept. 4 Aft.	7.50	2.37	4.62	6.83	3.33	3.15	7.12	3.75	3.03	6.88	2.12	4.28	7.083	2.893	3.770
" 7 Morn.	5.16	2.08	3.08	4.83	3.33	1.50	4.16	2.16	2.00	3.33	2.00	1.33	4.370	2.393	1.977
" 7 Aft.	4.58	1.16	3.42	4.45	1.83	2.62	4.16	1.75	2.41	6.00	1.42	4.58	4.798	1.540	3.258
" 9 Morn.	4.25	1.58	2.67	3.75	2.75	1.00	4.33	2.33	2.00	3.92	2.41	1.51	4.063	2.268	1.795
" 9 Aft.	3.17	1.33	1.84	4.41	2.21	2.20	4.58	2.71	1.87	3.17	2.75	0.42	3.833	2.250	1.583
" 11 Morn.	4.50	1.30	3.20	4.75	2.70	2.05	5.42	3.0	2.42	5.10	3.10	2.00	4.943	2.525	2.418
" 11 Aft.	3.50	1.41	2.09	3.58	2.58	1.00	3.67	2.58	1.09	3.60	2.08	1.52	3.588	2.163	1.425
" 17 Morn.	4.17	1.17	3.00	3.82	1.42	2.40	4.0	2.08	1.92	3.82	1.75	2.07	3.953	1.605	3.248
" 17 Aft.	3.82	1.82	2.00	3.33	1.43	1.90	2.92	1.30	1.62	4.58	1.33	3.25	3.663	1.470	2.128
" 21 Morn.	4.08	2.58	1.50	5.07	2.33	3.34	4.58	3.07	0.91	4.08	2.92	1.16	4.603	2.875	1.765
" 21 Aft.	4.75	2.83	1.92	4.90	3.92	0.98	3.30	1.75	1.55	3.58	2.17	1.41	4.133	2.668	1.465
Mean →	4.498	1.785	2.667	4.575	2.530	2.013	4.385	2.462	1.893	4.369	2.186	2.139	4.457	2.241	2.178

of treatment are shown in three columns at the extreme right, and the mean values for each treatment irrespective of 'occasion' in the lowest row of the table.

It will be seen that in no case has starch quite disappeared from the covered leaflets (the values for the average tone being in all cases greater than 1). Comparison of the values for starch production depends, therefore, very largely on the relation between increments in the tone scale and increment in starch content. We have seen that the tone scale forms at least an approximation to the required scale, and the preliminary analysis of the results will therefore be carried out on the figures of Table III. The general results will, however, be checked by an analysis of the results on the basis of percentage of black scale (see p. 340).

*Statistical Analysis of the Variation in Starch Production.*

The data for starch production form a  $4 \times 11$  table (four manurial treatments compared on eleven 'occasions'), and for data of this form R. A. Fisher has developed a very valuable method of analysis—the analysis of variance.<sup>1</sup> The method enables one to estimate the amount and the significance of the contribution made by 'manurial treatment' on the one hand and by 'occasion' on the other to the total variation of the set of observations. This analysis is shown in Table IV.

TABLE IV.

*Analysis of Variance of Starch Production.*

	<i>Sum of Squares.</i>	<i>Degrees of Freedom.</i>	<i>Variance.</i>	<i>Log<sub>e</sub> Variance.</i>
Total	40.5219	43		
Manuring	3.84495	3	1.28165	0.24818
Occasion	22.42560	10	2.24256	0.80762
Differential	14.25135	30	0.475045	0.255655

The total variation is represented by 40.5219, which is the sum of the squares of the deviations of each of the forty-four observed values from their mean. The contribution to this total made by manuring, 3.84495, represents eleven times the sum of the squares of the deviations of the mean of each manurial treatment from the general mean. The number of degrees of freedom available for this contribution is three (one less than the number of manurial treatments). Similarly, the sum of squares due to 'occasion' is four times the sum of the squares of the deviations of the mean of each of the eleven 'occasions' from the general mean, and ten degrees of freedom are available. The remaining fraction of the total sum of squares, 14.25135 ( $= 40.5219 - 3.84495 - 22.42560$ ), represents differential behaviour of the

<sup>1</sup> R. A. Fisher: *Statistical Methods for Research Workers*, London and Edinburgh, 1925.

different manurial treatments on different occasions, and to it are assigned the remaining thirty degrees of freedom. The mean square or variance (= sum of squares/degrees of freedom) is shown in the third column, and it will be seen that the variances due to 'occasion' and to manuring are much greater than that due to differential response. It remains to test whether the relative magnitudes of these variances indicate a significant effect of 'occasion' independent of manuring, and of manuring independent of 'occasion', upon starch production, or whether a chance combination of differential effects could have given rise to the observed figures. The standard of comparison developed by Fisher for this purpose is half the difference between the natural logarithms of the variances to be compared. For the comparison between the variances due to manuring and to differential effects, this value,  $Z$ , is equal to 0.49621; for the similar comparison between 'occasion' and differential effects  $Z$  is equal to 0.77598. For the first case, the value of  $Z$  required for  $P = 0.05$ , i.e. for the probability that a difference as great as that observed shall occur by chance not more than once in twenty trials, is 0.5364. In the second case the value of  $Z$  required is lower; since ten instead of three degrees of freedom are available for estimating the larger variance, the value of  $Z$  required is 0.4055.

The contribution made by manuring just fails to reach a significance of  $P = 0.05$ , while the contribution of 'occasion' is undoubtedly significant. The relatively small contribution made by manuring independent of 'occasion' to the total variation is due, as the lower row of Table III shows, to the fact that the two potash manures containing chlorides have hardly any effect on the mean starch production. The potassium sulphate plot alone shows any superiority over the potash-starved plot. This single manurial effect is swamped in the general analysis by the absence of effect from the other two manurial treatments. For an estimate of the significance of this effect we may calculate from the differential variance the standard deviation of a comparison between the means of any two manurial treatments.

$$\sigma = \sqrt{\frac{0.475045 \times 2}{11}} = 0.29389.$$

$$2\sigma = 0.58778.$$

A difference between means exceeding 0.58778 may therefore be regarded as significant. The observed differences are  $K_2SO_4 - KCl = 0.528$ , - no potash = 0.654, - P.M.S. = 0.774, so that the  $K_2SO_4$  plot is definitely superior to the 'no potash' and the P.M.S. plot, but there is no significant difference between KCl, 'no potash', and P.M.S.

In the above analysis the variance due to differential response has in effect been used as an estimate of random variance. It is *a priori* probable that the differently manured plots will respond differently on different occasions, but to estimate the importance of this factor we require an estimate of

the random variance due to sampling. From the data for the individual leaflet we may make an estimate of the random variance to be expected in a set of observations derived from samples of six leaflets. The essential data are given in Table V.

TABLE V.

Plot.	Variance of Individual Leaflets.		Variance of the Difference = Starch Production.	Variance of Mean Starch Production.
	Exposed.	Covered.		
K <sub>2</sub> SO <sub>4</sub>	2.076	1.029	3.105	0.5175
No potash	3.199	2.416	5.615	0.9358
P.M.S.	2.051	2.012	4.063	0.6772
KCl	1.851	1.229	3.080	0.5133
Mean	2.294	1.6715	3.966	0.6611

It will be seen that, assuming no correlation between the covered and the exposed leaflets, the random variance calculated, 0.6611, is greater than the differential variance observed (Table IV). There is, however, a strong correlation between paired leaflets which the above calculation neglects. Direct calculation of the variance of the difference between paired leaflets, one covered and one exposed, is not possible, because, owing to the large number involved, the leaflets could not be kept in their pairs during boiling and decolorizing for the starch test. There are data, however, for six pairs of leaflets from each plot on September 10, from which the following coefficients of correlation between paired leaflets are calculated: K<sub>2</sub>SO<sub>4</sub> plot,  $r = +0.8094$ ; no potash plot,  $r = +0.8274$ ; P.M.S. plot,  $r = +0.8250$ ; KCl plot,  $r = +0.8163$ . For the whole set, twenty-four pairs,  $r = +0.9348$ . We may use the lower figure +0.8 for an approximate estimate of the variance of the difference between paired leaflets.

From the relation  $\sigma^2_{A-B} = \sigma^2_A + \sigma^2_B - 2\sigma_A\sigma_B r_{AB}$ , where  $A$  and  $B$  refer to the exposed and covered leaflets, if  $r_{AB} = +0.8$ , then  $\sigma^2_{A-B} = 0.1295$ . This is probably too low an estimate, but even if it were doubled the variance due to differential effects would still remain insignificantly greater. There is some evidence, therefore, for a significant differential effect of manure and 'occasion' upon starch production.

Returning now to the interpretation of the mean values for starch production for the different treatments, it is evident from the bottom row of Table III that the superior starch production of the potassium sulphate plot is associated rather with a decrease in the mean value of the covered leaflets than with an increase in the mean value of the exposed leaflets. The mean values for the exposed leaflets are indeed almost the same for all the plots. The data suggest—and the suggestion is confirmed, at least for the first half of the month, by the results of a few direct experiments on

translocation—that the rate of translocation of starch from the leaves is greater with the potassium sulphate manuring, less with the KCl and P.M.S. manuring, and least in the absence of potash. From Table III a comparison of the mean values for the covered leaflets at 12.30 (*circa*) and at 3.30 (*circa*) shows that by noon the  $K_2SO_4$  leaves have already a low starch content and show thereafter little further change. The two chloride plots, while relatively high at noon, fall in the afternoon towards the level of the  $K_2SO_4$  plots, while the potash-starved plot, which is as high as these at noon, falls but slowly during the next three hours.

*Average Covered Leaf Values.*

	$K_2SO_4$	No potash.	P.M.S.	KCl.
12.30 p.m.	1.742	2.506	2.628	2.432
3.30 p.m.	1.712	2.396	2.018	1.830

Again, the average of a number of samples of untreated leaflets taken about 9.30 a.m. shows the potash-starved plot still high in starch (6.08), while the other plots are low (KCl 5.08, P.M.S. 4.85,  $K_2SO_4$  4.7). The mean of a number of similar samples taken at 5 p.m. shows the plots in exactly the reverse order, though the differences between plot and plot are smaller.

The association of the higher figure for mean starch production with the lower mean starch content of the covered leaflet thus suggests three possibilities. (1) That during the three hours' exposure all leaflets, irrespective of treatment, can reach, but cannot exceed, a certain limit of starch content. (2) That the rate of starch production is lower the greater the amount of starch already in the leaf. (3) That within the manurial plots under observation a low rate of starch removal is associated with, though not of necessity causally related to, a low rate of starch production, both phenomena being due to an impaired protoplasmic mechanism. On either of the first two suppositions the superiority in starch production shown by the potassium sulphate plot would be ascribed to a superior rate of translocation of starch and would presumably not have been shown had all the leaflets been completely destarched before exposure. The first possibility is, however, excluded by an inspection of the mean values for the exposed leaflets on each of the eleven 'occasions'. They are far from constant, their standard deviation being greater than that for the covered leaflets, 0.9781 as against 0.5106. If the second supposition is correct there should be a strong negative correlation between the mean values for starch production on each occasion and the corresponding values for the covered leaflets. The correlation coefficients are (where starch production = 1, exposed leaflet = 2, covered leaflet = 3):

$$r_{12} = +0.7356$$

$$r_{13} = -0.0992.$$

The correlation with the covered leaflet is indeed negative, but is insignificantly small. The amount of starch left in the leaf thus contributes little to the variation in starch production on different occasions. This factor can therefore be of small importance in accounting for the mean difference in starch production between treatments. This becomes quite clear if, using the regression coefficient for the equation between starch production and covered leaflet value, we calculate from the mean covered leaflet value for each treatment the expected difference in mean starch production between treatments. Thus we have

	<i>Expected.</i>	<i>Observed.</i>	<i>Difference.</i>
$K_2SO_4 - KCl =$	0.0584	0.528	0.4696
$K_2SO_4 - \text{no potash} =$	0.1085	0.654	0.5455
$K_2SO_4 - \text{P.M.S.} =$	0.0986	0.774	0.6754
Mean $K_2SO_4 - \text{rest} =$	0.0892	0.652	0.5628

Thus the superior starch production of the  $K_2SO_4$  plot, while apparently associated with, is not wholly due to a more rapid rate of translocation of starch from the leaf. The mean difference in rate of starch production may well be due to differences in the efficiency of the photosynthetic mechanism.

#### *Analysis of the Variance due to 'Occasion'.*

In the analysis of variance of starch production ten degrees of freedom were assigned to 'occasion'. It will be interesting to see whether the greater part of this variance can be ascribed to the varying intensity of one or two main factors such as might influence rates of carbon assimilation. Two factors suggest themselves: (1) intensity of radiation, (2) age, since we are dealing with the phase of growth in which 'ripening off' is beginning. Radiation data are available from the Callendar Recorder charts, and have been calculated as average intensity of total radiation during the period of exposure of the leaflets.<sup>1</sup> Age may be reckoned in days, the first day of the experiments, September 4, counting as one (Fig. 2). The correlations between these factors and the mean starch production (irrespective of treatment) are as follows (1 = starch production, 2 = radiation, 3 = age):

$$\begin{aligned} r_{12} &= +0.23629 & r_{12.3} &= +0.58363 \\ r_{13} &= -0.47837 & r_{13.2} &= -0.67927 \\ r_{23} &= +0.45828. \end{aligned}$$

It will be seen that the partial correlation coefficients are of the sign and approximately of the order that would be expected if we were dealing with carbon assimilation. It remains to estimate their significance. The

<sup>1</sup> The values given for radiation in Fig. 2 represent millimetres on the Callendar Recorder charts. One millimetre corresponds to 0.0092 calorie per cm.<sup>2</sup> per min.



calculation involves the fitting of two constants derived from the data, hence the number of degrees of freedom remaining is 8 ( $10-2$ ). For this number of degrees of freedom the levels of significance are: <sup>1</sup>

$$\text{for } P = 0.1 \quad r = 0.5494$$

$$P = 0.05 \quad r = 0.6319.$$

The negative correlation with age is therefore quite significant, while the

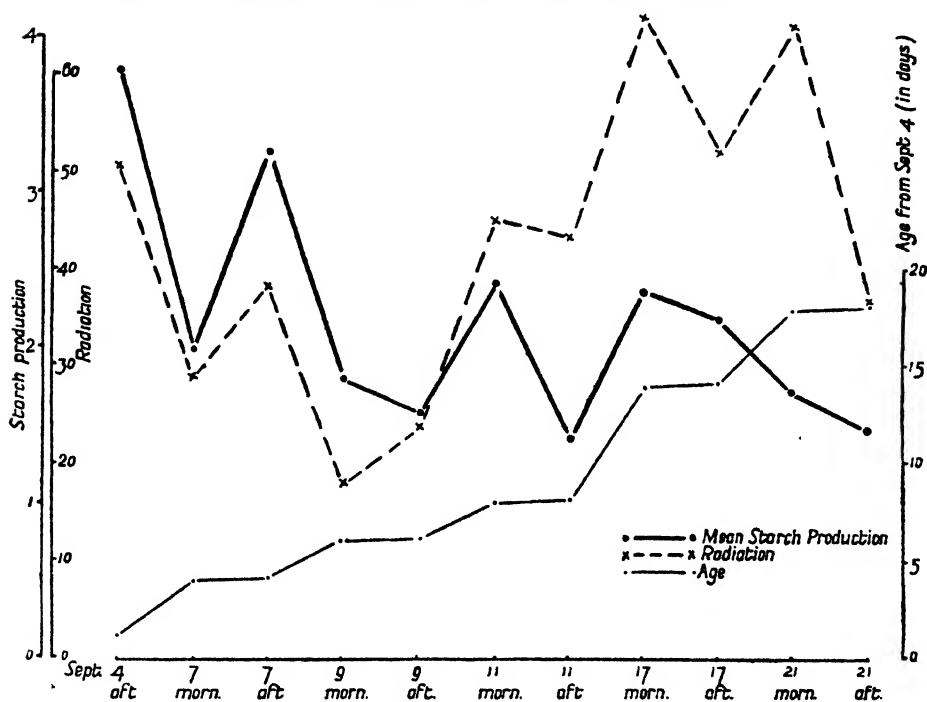


FIG. 2. Graph showing the relationship between mean starch production, radiation, and age of leaf for the period Sept. 4 to 21.

positive correlation with radiation is, owing to the small number of degrees of freedom available, less certain ( $P$  lies between 0.1 and 0.05). The two factors together, however, account for more than 60 per cent. of the total sum of squares due to 'occasion', as Table VI shows.

TABLE VI.

*Analysis of Variance due to 'Occasion'.*

	Sum of Squares.	Degrees of Freedom.	Variance.	Log <sub>e</sub> Variance.
Total	22.42560	10	2.24256	0.80762
Fit to regression on radiation and age	14.02060	2	7.01030	1.94738
Deviation	8.40504	8	1.5063	0.40954

<sup>1</sup> Fisher, loc. cit., Table V A.

Comparing the variance due to radiation and age with the remainder, we have  $Z = 0.76892$ . For  $P = 0.05$   $Z$  should be  $0.7475$ ,<sup>1</sup> so that the contribution made by these two factors to the total variance is significant.

*Differential Response of Different Treatments.*

In view of the suggestion (see p. 336) of a significant differential response of manurial treatment and 'occasion' the correlations with radiation and age for each plot separately will be of interest.

These are (where 1 = starch production, 2 = radiation, 3 = age):

$K_2SO_4$	$r_{12.3} = +0.47388$	P.M.S.	$r_{12.3} = +0.18442$
	$r_{13.2} = -0.73641$		$r_{13.2} = -0.64938$
No potash	$r_{12.3} = +0.62740$	KCl	$r_{12.3} = +0.46645$
	$r_{13.2} = -0.39012$		$r_{13.2} = -0.50104$

The correlations which reach a level of significance,  $P = 0.05$ , are underlined. It is interesting to note that the plot deficient in potash gives the highest positive correlation with radiation and the lowest negative correlation with age. The values for starch production (Table III and Fig. 1) show that the superiority of the potassium sulphate plot over the 'no potash' plot diminishes as radiation increases or as the plants age. So far as concerns radiation, this effect is in the direction that might be expected if we were dealing with rates of carbon assimilation, since there is evidence from the work of Briggs<sup>2</sup> that deficiency in mineral salts diminishes the efficiency of the photochemical and chemical phases of photosynthesis, and the effect of this diminished efficiency upon the rate of photosynthesis under natural conditions of carbon dioxide supply will be greater the lower the intensity of radiation.

*Analysis of the Experimental Results on the Percentage of Black Scale.*

So far, the analysis of the results has been made on the basis of the tone scale used in grading the leaflets. Adjusting the individual observations to the scale of percentage of black, we have the results summarized in Table VII.

As will be seen from the analysis of variance, the effect of manuring and of 'occasion' are both significant, and while the mean difference in starch production between the three plots, 'no potash', P.M.S., and KCl, remains insignificant, the superiority of the  $K_2SO_4$  plot is more marked than before. Again, the mean values for the exposed leaflets vary very little from plot to plot, so that the greater starch production of the  $K_2SO_4$  plot depends on the much lower mean value of its covered leaflets. The correlations between

<sup>1</sup> Fisher, loc. cit., Table V A.

<sup>2</sup> G. E. Briggs: Proc. Roy. Soc., B., vol. xciv, p. 28, 1922.

mean starch production on each occasion and mean values for the covered and exposed leaflets are as follows (where 1 = starch production, 2 = exposed leaflet, 3 = covered leaflet):  $r_{12} = +0.44146$ ,  $r_{13} = -0.59863$ .

TABLE VII.

*Scale of Percentages of Black.*

*Mean of all Four Treatments.*

Date.		Exposed.	Covered.	Starch Production.
Sept. 4	Aft.	92.69	45.48	42.5
" 7	Morn.	70.10	39.95	30.15
	Aft.	74.84	22.35	52.49
" 9	Morn.	66.50	42.24	24.26
	Aft.	63.67	38.18	25.49
" 11	Morn.	74.96	42.35	32.62
	Aft.	59.02	36.18	22.84
" 17	Morn.	66.31	25.09	41.22
	Aft.	61.88	21.34	40.54
" 21	Morn.	69.53	45.08	24.45
	Aft.	65.36	42.66	22.70

*Mean of the Eleven Values for each Treatment.*

	Exposed.	Covered.	Starch Production.
K <sub>2</sub> SO <sub>4</sub>	70.47	25.56	44.398
No potash	70.32	41.26	28.761
P.M.S.	69.19	41.806	24.014
KCl	68.149	37.157	30.457

*Analysis of Variance.*

	Sum of Squares.	Degrees of Freedom.	Variance.	Log <sub>e</sub> Variance.
Total	8798.42	43		
Manuring	2086.62	3	695.54	6.55469
Occasion	4066.80	10	406.68	6.00803
Differential	2645.00	30	88.17	4.47926

$Z$  for 'manuring' = 1.03772. For  $P = 0.05$   $Z$  is 0.5364.

$Z$  for 'occasion' = 0.76439. For  $P = 0.05$   $Z$  is 0.4055.

2  $\sigma$  for a comparison between means of two manurial treatments = 8.007.

The negative correlation between the value for the covered leaflet and starch production is large enough to account for a considerable portion of the observed difference in starch production between different treatments. The following table shows the observed difference between the K<sub>2</sub>SO<sub>4</sub> plot and the other three plots, the difference to be expected from the mean values of the covered leaflets for each treatment, and also the remainder.

Plots.	Observed Difference.	Expected Difference.	Remainder.
K <sub>2</sub> SO <sub>4</sub> — KCl	13.94	7.67	6.27
K <sub>2</sub> SO <sub>4</sub> — no potash	16.64	10.38	6.26
K <sub>2</sub> SO <sub>4</sub> — P.M.S.	17.38	10.74	6.64
Mean			6.39

Thus the individual differences between plots remaining after this allowance is made amount to little more than 1.5 times their standard

deviation, and are not significant. The mean difference between the  $K_2SO_4$  plot and the other three is, however, still just significant.

As before, the variance due to 'occasion' can be partly ascribed to varying intensity of radiation and to ageing of the leaflets. The correlation coefficients are of the same sign as before, but of a somewhat smaller order, and do not attain the required level of significance.

$$r_{12.3} = +0.5075 \qquad r_{13.2} = -0.50379.$$

The differential response of the  $K_2SO_4$  and the 'no potash' plots is again evident, and is of the same nature as before.

$$\begin{array}{ll} K_2SO_4 \ r_{12.3} = +0.42005 & \text{No potash } r_{12.3} = +0.64876 \\ r_{13.2} = -0.64224 & r_{13.2} = -0.30071 \end{array}$$

Thus the main features shown by the analysis of the results on the scale of tone values are again found on the scale of percentage of black. We may, however, still keeping to the percentage of black scale for the exposed and covered leaflets, attempt the elimination of the effect of variations in the covered leaflet values by calculating starch production as a percentage of the total possible increase in percentage of black, i.e. as  $\frac{100 \times (\text{Exposed} - \text{Covered})}{100 - \text{Covered}}$ . On this basis the mean values for 'occasion' and for treatment are as in Table VIII.

The variances due to 'occasion' and to manuring are both significantly greater than the differential variance, and the mean difference between the  $K_2SO_4$  plot and each of the others is also significant. That the method of calculation has to a large extent eliminated the effect of variation in covered leaflet values upon mean starch production is shown by the correlation coefficients (where 1 = mean starch production, 2 = exposed leaflet, 3 = covered leaflet):

$$r_{12} = +0.7863 \qquad r_{13} = -0.1611.$$

The small negative correlation which still remains between covered leaflet value and starch production does not appreciably affect the comparison of the means of manurial treatments, as the following calculation of differences not due to variation in mean covered leaflet value shows:

$$\begin{array}{lll} K_2SO_4 - \text{no potash} & = & 8.054 \text{ instead of } 10.58 \\ K_2SO_4 - \text{P.M.S.} & = & 9.596 \quad \text{,,} \quad 12.21 \\ K_2SO_4 - \text{KCl.} & = & 11.196 \quad \text{,,} \quad 13.06 \end{array}$$

The contributions of radiation and age to the variance due to 'occasion' are now greater than before (1 = starch production, 2 = radiation, 3 = age):

$$^1 r_{12.3} = +0.66172 \qquad r_{13.2} = -0.69611,$$

both correlations being now statistically significant.

The differential response of the  $K_2SO_4$  and 'no potash' plots to radiation and age is of the same order as before :

$$\begin{array}{ll} K_2SO_4 \ r_{12.3} = + 0.47521 & \text{No potash } r_{12.3} = + 0.72587 \\ r_{13.2} = - 0.70971 & r_{12.2} = - 0.67453 \end{array}$$

TABLE VIII.

*Starch Production as a Percentage of the Total Possible Increase in Percentage of Black of Scale.*

Date.			Mean of all Treatments.	Mean of each Treatment.			
				$K_2SO_4$ .	No potash.	P.M.S.	KCl.
Sept.	4	Aft.	77.33				
"	7	Morn.	50.03				
		Aft.	66.48	59.21	48.63	47.00	46.15
"	9	Morn.	40.88				
		Aft.	40.10				
"	11	Morn.	55.30				
		Aft.	34.18				
"	17	Morn.	53.60				
		Aft.	51.50				
"	21	Morn.	44.10				
		Aft.	39.43				

Analysis of Variance.				
	Sum of Squares.	Degrees of Freedom.	Variance.	Log <sub>e</sub> Variance.
Total	10802.06	43		
Manuring	1213.08	3	404.36	6.00230
Occasion	6556.84	10	655.684	6.48568
Differential	3032.14	30	101.071	4.61579

$$\begin{array}{ll} Z \text{ for 'manuring'} = 0.69325. & \text{For } P = 0.05 \text{ } Z \text{ is } 0.5364. \\ Z \text{ for 'occasion'} = 0.93495. & \text{For } P = 0.05 \text{ } Z \text{ is } 0.4055. \\ 2 \sigma \text{ for a comparison between means of two treatments} = 8.5742. \end{array}$$

### CONCLUSIONS.

It thus appears that in healthy leaflets of potato plants during the later stages of growth the rate of starch production as measured by Sachs's iodine test varies significantly with variations in potash manuring and with certain periodic factors, of which intensity of solar radiation and age have been shown to be important. During this period of growth, potash manures containing chlorides have not on the average produced any significant improvement in the rate of starch production, but manuring with potassium sulphate has produced a marked improvement. This superiority of the plants treated with potassium sulphate is associated with a more rapid translocation of starch from the leaflets, but does not appear to be wholly due to that factor. In addition to a certain average difference in rate of starch production between differently treated plots there appears to be a significant difference in response to environmental conditions and to age. As between the potassium sulphate treatment and the 'no potash' treatment part of this differential response may be due to a closer association, on the

potash-starved plot, between rate of starch production and intensity of radiation, and part also to a more rapid deterioration with increasing age on the potassium sulphate plot, which starts initially at the higher level.

The general conclusions do not appear to be appreciably affected by the method of expressing the intensity of colour developed in the iodine test for the starch in the leaflets. It seems probable, however, that the units of the colour scale actually used for grading the leaflets (Colour Scale Ridgway 59<sup>||||</sup>) do form a scale which gives an approximate measure of changes in starch production content, provided an intense violet black colour is not reached. It is hoped in further work to make a comparison between the iodine test and chemical determinations of the starch content of leaflets. Meanwhile, the elements of order which even these preliminary observations have shown upon analysis justify the belief that the Sachs test may prove of great service for field studies in crop physiology.

#### SUMMARY.

1. Field observations have been made upon the rate of starch production in leaflets of potato plants grown without potash manure and also with three different types of potash manures, Sachs's iodine test being employed. The observations relate to the later period of growth of the plants.

2. The use of a standard scale of colour tones for grading the colour intensities developed in the leaf by the iodine test is discussed. Two alternative methods for giving numerical values to these colour intensities are put forward: (*a*) the use of the actual units of the tone scale; (*b*) the use of a scale of black derived from the colour-wheel analysis of the tone scale.

An analysis of the experimental data for starch production, using either of these methods, leads to the same general conclusions, but evidence is presented which suggests that the units of the particular standard tone scale employed form the better approximation to a measure of starch content. The tone scale found to be satisfactory is tone scale 59<sup>||||</sup> of Ridgway's 'Colour Standard and Nomenclature'.

3. The observed variation in starch production is analysed statistically, and it is shown that while the application of potash manures containing chlorides has not appreciably improved the rate of starch production, the application of potassium sulphate has done so. At the same time also the rate of translocation of starch from the leaflets on the potassium sulphate plot is increased.

The writer wishes to record his special indebtedness to Mr. Catley, of the University of Sydney, and Mr. Dawson, of the University of Manchester, who began the work with him, and were almost entirely responsible for the series of observations made. Thanks are also due to Mr. Eden for assistance with the sampling and in other ways.

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## CLVI. THE DETERMINATION OF CELLULOSE IN STRAWS.

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*(Received August 20th, 1930.)*

FOR the determination of cellulose in straws the standard method of Cross and Bevan [1918] is the most reliable. However, it suffers from three disadvantages, which are (1) the inconvenience of working with gaseous chlorine, (2) the lengthy extraction with sodium sulphite solution which is required to remove the lignone chloride, and (3) the difficulty of chlorinating a number of samples simultaneously.

A method of determining cellulose in straws is described in this paper, in which the straw is chlorinated by means of sodium hypochlorite in alkaline solution; it is first necessary to boil the straw with dilute acid and alkali in order to bring it into a condition in which it is amenable to hypochlorite treatment. After boiling with acid and alkali the lignin present in the extracted straw is chlorinated and dissolved at the same time while the cellulose remains unattacked owing to its stability in presence of neutral or alkaline hypochlorites in dilute solution. Two such chlorinations completely remove the lignin, leaving a white or cream-coloured product which retains the whole structure of the straw. This product consists of cellulose intimately associated with xylan and in character is identical with the cellulose of Cross and Bevan. One unimportant difference exists between the two methods, viz. the xylan fraction expressed as a percentage of the chlorination product ("cellulose") is less in the case of the hypochlorite method than in that of Cross and Bevan. Actually more "cellulose" is obtained by the latter procedure so that when the xylan content of the chlorination product is taken into account the yields of true cellulose by the two methods are identical. Using hypochlorite it has been found possible for one worker to carry out from 12 to 16 cellulose determinations in a day.

One obvious advantage which the use of hypochlorite offers is the preparation of straw celluloses in a form suitable for structural or chemical examination. To obtain a theoretical yield of cellulose from 200 or 300 g. of straw is quite simple. A preparation of this magnitude involving the use of gaseous chlorine would be tedious and unpleasant.

The suitability of the hypochlorite method for the determination of wood cellulose is now being studied and the results will be given in a later paper.



## EXPERIMENTAL.

*Solutions required.* (1) Sodium hypochlorite, commercial, 15 % free chlorine (Baird and Tatlock, Ltd., London). This should be stored in a cool dark place. The strength of this solution varies from 14 to 19 %, but unless the free chlorine lies outside this range it is not necessary to make allowance for the variation in the volumes of hypochlorite taken for the chlorination. The free chlorine may be determined as follows: 5 cc. of hypochlorite are made up to 500 cc. with water; 10 cc. of the dilute solution are treated with 10 cc. of 5 % acetic acid and 5 cc. of 10 % potassium iodide. The iodine liberated is titrated with *N*/20 sodium thiosulphate using a starch indicator. Then:

$$\% \text{ free chlorine} = \text{cc. of } N/20 \text{ thiosulphate} \times 1.775.$$

(2) Sodium hydroxide, 10 % solution.

(3) 10 % hydrochloric acid; 30 cc. conc. hydrochloric acid made up to 100 cc. with water.

(4) 2 % hydrogen peroxide: 10 cc. of 20 vol. hydrogen peroxide made up to 100 cc. with water.

*Standard method for the estimation of cellulose in straws.* 2 g. of very coarsely pulverised straw in a beaker are brought to the boil with 100 cc. of water and 10 cc. of 10 % NaOH. The beaker is then immediately placed in a boiling water-bath for 5 min. and the solution is poured on to a piece of white poplin cloth stretched over a 2½ inch Büchner funnel. When the liquid has been sucked off the straw is washed with water, the washings being poured on to the cloth each time. The straw is then transferred from the cloth to the beaker by means of a wide jet of water, and the volume is made up to approximately 100 cc. Then 10 cc. of 10 % HCl are added, the mixture is brought to the boil and the beaker placed in a boiling water-bath for 5 min. The straw is filtered and washed as before. The extraction of sugars, starch, and hemicelluloses is completed by a second alkali and acid treatment carried out in exactly the same manner as the first.

The extracted straw is made up to about 100 cc. and 5 cc. of sodium hypochlorite solution are added. This mixture is allowed to stand for 20 min. in a cool place away from bright sunlight. During this time the reaction of the solution must remain alkaline to brilliant yellow or litmus paper: sodium hydroxide solution is added if necessary. The straw is then filtered, washed, transferred to the beaker and chlorinated a second time for 20 min. It is next filtered through cloth, well washed with cold water, then with 100 cc. of 2 % hydrogen peroxide, and then with boiling water. Finally the residual cellulose is washed back into the beaker and then on to a weighed, oven-dried Gooch crucible. It will be found that rapid filtration without loss of cellulose is attained by using a cotton disc of lawn or organdie in place of an asbestos filter. A furfuraldehyde determination is made on the dried and weighed hypochlorite cellulose by the Tollens method [1902] using the Kröber factor [1900] for converting the phloroglucide yield to furfuraldehyde or xylan.

*Effect of the hypochlorite method on the yield of cellulose and furfuraldehyde.*

To test the percentage recovery of cellulose in the method described above and the amount of oxycellulose formed, a pure cotton cellulose was prepared in the following manner. A half-pound hank of the best American cotton was gently boiled with successive quantities of 1 % sodium carbonate for 4 hours, acidifying between each boil with 0.5 % HCl. After four such treatments the cotton was well washed with water and then bleached twice with 0.25 % sodium hypochlorite solution. The cotton was kept out of contact with air during the bleaching. It was thoroughly washed with water, then with 0.1 % HCl and again with water until the  $p_H$  of the washings and of the water used was identical. After the excess of water had been squeezed out of the cotton it was dried at 100°. The purified cellulose gave a yield of 0.29 % furfuraldehyde which corresponds to 0.46 % of anhydroxylose. It is not suggested that the furfuraldehyde-yielding substances in cotton cellulose consist entirely of xylose units but the figure is given for comparison with the anhydroxylose in straw cellulose.

Table I shows the effect on the pure cellulose of (a) two chlorinations with 5 cc. NaOCl and 100 cc. of water, each of 20 min.; (b) two extractions with 1 % NaOH and 1 % HCl, followed by two chlorinations for 20 min. as in the method described for straws.

Table I.

Treatment	(a)	(b)
Recovery of cellulose %	97.28	97.01
Anhydroxylose in cellulose %	0.59	0.43

It may therefore be concluded that neither treatment has an appreciable oxidising effect on the cellulose. Figures of a similar order are obtained when pure cellulose is chlorinated according to the Cross and Bevan method.

The yield of cellulose obtained by the Cross and Bevan method and by the hypochlorite method will now be compared. The latter procedure, as has already been pointed out, always seems to give about 2 or 3 % less of the chlorination product than the standard method. But when allowance is made for the larger amount of xylan (shown by the higher furfuraldehyde yield) invariably present in the Cross and Bevan cellulose it is at once seen from the figures in Table II that the amount of true xylan-free cellulose obtained is the same by both methods.

Table II.

Method	"Cellulose" in 100 g. oat straw g.	Furfurald. yielded by 100 g. "cellulose" g.	Furfurald. yielded by "cellulose" in 100 g. straw	Xylan equivalent of furfurald. in "cellulose"	Pure cellulose in straw %
Cross and Bevan	48.92	12.30	6.02	9.33	39.59
Hypochlorite	47.50	11.07	5.26	8.15	39.59

Further examples showing the results of cellulose estimations by the hypochlorite method are given in Table III.

Table III.

Straw	Hypochlorite "cellulose" %		Furfurald. in hypochlorite cellulose %		Anhydro- xylose equivalent to furfurald. %		Anhydro- xylose in hypochlorite cellulose in 100 g. straw		Pure cellulose in straw %	
Rye	56.36	55.66	14.56	14.20	22.55	22.01	12.71	11.3	43.65	43.36
Barley	50.45	49.25	11.05	11.24	18.33	18.05	9.25	8.89	41.20	40.36
Oat from straw filter fed with nitrogenous soln. for 23 days	51.10	51.21	10.94	11.22	16.95	17.39	8.49	8.91	41.61	42.20
Oat from straw filter fed with water alone for 53 days	54.01	52.45	12.77	10.53	19.78	16.21	10.33	8.50	43.33	43.95

*Preparation of cellulose from straws.* The examination of reasonably pure celluloses from straws is obviously of importance in connection with X-ray analysis, the morphology of the plant, and its chemical constitution. Hitherto such examination has been somewhat restricted owing to the difficulty of preparing large samples of the cellulose in a reasonably pure and unchanged condition. However, with the hypochlorite method very little modification of the proportions of the reagents used in the estimation is required for the isolation of larger quantities of cellulose. The following method has proved satisfactory for oat straw. 178.5 g. of dry straw were mixed with 7 litres of boiling water containing 100 cc. of concentrated HCl and left in a boiling water-bath for 10 min. The mixture was then strained through a cloth and the residual straw added to 7 litres of boiling water containing 15 g. of NaOH. After standing in the boiling water-bath for 10 min. the aqueous portion was poured off through a cloth and then chlorinated for 20 min. with 200 cc. of 15 % NaOCl. The straw was filtered off, washed and again chlorinated with the same quantity of hypochlorite. After washing the cellulose first with cold, then with hot water, it was washed with 2 litres of 0.5 % hydrogen peroxide. The use of hydrogen peroxide ensures complete decomposition of the hypochlorite. Finally the cellulose was washed free from hydrogen peroxide with cold and hot water. In this manner 93 g. of cellulose were obtained, equivalent to a 52.1 % yield on the weight of straw taken. An analysis of the sample of oat straw showed that it contained 52.2 % of cellulose, which gave a furfuraldehyde yield of 11.1 %.

## SUMMARY.

1. The cellulose in straw is readily determined by treating the straw with hot dilute alkali and acid and then with cold hypochlorite solution.
2. The product obtained is practically identical in character with the Cross and Bevan chlorination product, except that it contains slightly less xylan than the latter. When due allowance is made for the xylan present the

percentage of pure cellulose found in a straw is the same by the Cross and Bevan and the hypochlorite method.

3. The treatment referred to in 1 has a negligible effect on pure cellulose.

4. The hypochlorite method of chlorinating has the following points in its favour.

(a) From 12 to 16 cellulose determinations can be carried out in a day by one worker.

(b) Large scale preparations of straw cellulose are possible without inconvenience.

(c) The cellulose can be prepared in quantitative yield.

The writer welcomes this opportunity of recording his thanks to Dr A. G. Norman of the Rothamsted Experimental Station for carrying out the Cross and Bevan determinations of cellulose given in this paper and for his helpful criticism.

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# THE INFLUENCE OF MANURIAL TREATMENT ON THE BAKING QUALITY OF ENGLISH WHEAT.

## I. A QUALITY STUDY OF THE ROTHAMSTED BROADBALK WHEATS.

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(With Plates III and IV.)

IN any enquiry as to the possibility of influencing the quality of English wheat by means of manurial treatment the Broadbalk Field of the Rothamsted Experimental Station offers an unique opportunity in certain directions. As is well known, the respective plots of the Broadbalk Field have received constant manurial treatment under the continuous growth of wheat for a lengthy period of approximately ninety years. The dosages, moreover, have been very heavy. The soil of each plot, and the wheat grown upon it, might therefore be expected to show to an unusually marked degree the effect of the particular fertiliser to which it has been subjected.

Precise records of the yields of wheat and straw from the various Broadbalk plots throughout the years have been kept at Rothamsted, but the samples have not been tested in the bakehouse except in 1903 and 1904 when trials were made on the crops of those years by Dr A. E. Humphries<sup>(8)</sup> and the Home-grown Wheat Committee of the National Association of British and Irish Millers. We have received each season since 1926 bushel samples of certain of the Broadbalk wheats. These samples have been milled on our experimental mill, and the straight-run, or 100 per cent., flours tested in the bakehouse according to our usual procedure.

It is unnecessary here to describe in detail the Broadbalk wheat-growing. It will suffice to recall the manurial treatments and give the yield of wheat per acre and the nitrogen contents of the grain, crop by crop, for the various plots we have tested. This is done in Table I. It will be noticed that one or two plots have been dealt with in certain seasons and not in others. The plots of particular interest from the point of view of baking quality are those featuring nitrogenous manuring in different



amounts and forms. These include Plots 2, 3, 6, 7, 8, 9 and 16, and it is obvious from the information as to manurial treatment in Table I that the following comparisons should be of particular interest:

- (a) 6, 7 and 8,
- (b) 9 and 16,
- (c) 6 and 9,
- (d) 7 and 16.

Table I shows that the nitrogen contents are increased consistently only by the heaviest nitrogenous treatments. The lowest nitrogenous treatments (cf. Plots 6 and 9 which show closely similar nitrogen contents) do not significantly raise the nitrogen content of the wheat as compared with the unmanured, and they actually lower the nitrogen content in comparison with that of the plots dressed with complete minerals only. In this connection it must be remembered that the yield per acre of grain is increased by the lowest nitrogenous treatments. It will be noticed that the farmyard manure in the dosage applied has as great an effect upon nitrogen content as the heavier dosages of the artificial nitrogenous fertilisers.

The type of wheat grown throughout these trials was Red Standard in 1926, 1927 and 1928 and Squarehead's Master in 1929. In 1926 a change was made in the hitherto unbroken routine of cultivation. Up to that year the wheat had been grown continuously and the unrested land had become exceedingly dirty in the agricultural sense. Conditions were so bad that in 1926 and 1927 one half of the field was fallowed and the 1928 and 1929 crops were grown on these fallowed portions. As a result yields were increased, and the appearance and condition of the grain were improved almost out of recognition. The change in procedure can be discerned in Table I: the nitrogen contents of the 1928 and 1929 crops tend to be consistently lower than those of the preceding seasons; the yields on the other hand are strikingly higher.

In Table II are given nitrogen contents and "maltose figures" for the flours of the four seasons' crops. The maltose figure is the percentage of reducing sugar found after incubating 20 gm. of flour with 160 c.c. of water for 1 hour at 27° C. The figure includes the reducing sugar originally present and that formed during incubation. The figure is a good indication of soundness of grain, since even a slight degree of sprouting would cause a marked increase in diastatic activity and hence in maltose figure. In most normally sound wheats the maltose figure as conventionally determined is usually below 2 per cent. It is evident from Table II that

Table I. *Details of manurial treatment, yield of grain per acre and nitrogen contents of the Broadbalk wheats for the four seasons 1926, 1927, 1928 and 1929.*

Plot	Manurial treatment	1926 crop			1927 crop			1928 crop			1929 crop		
		Yield of grain* (bushels per acre)	Nitrogen content† (%)	Yield* (bushels)	Nitrogen content (%)	Yield* (bushels)	Nitrogen content (%)	Yield* (bushels)	Nitrogen content (%)	Yield* (bushels)	Nitrogen content (%)	Yield* (bushels)	Nitrogen content (%)
2 A	Farmyard manure (14 tons per acre)	...	...	19.5	2.08	41.1	1.84	23.3	—	17.7	1.54	23.3	—
2 B	Farmyard manure (14 tons)	...	...	24.2	1.97	48.4	1.96	30.0	1.75	30.0	1.75	30.0	1.75
3	Unmanured since 1839	...	...	6.9	1.74	27.9	1.56	9.1	1.51	9.1	1.51	9.1	1.51
5	Complete minerals‡: no N	...	...	6.5	1.70	35.2	1.70	9.1	—	9.1	—	9.1	—
6	Ditto: + 206 lb. $\text{Am}_2\text{SO}_4$ §	...	...	12.5	1.63	47.3	1.62	17.7	1.54	17.7	1.54	17.7	1.54
7	Ditto: + 412 lb. $\text{Am}_2\text{SO}_4$	...	...	21.5	1.93	67.4	1.70	20.9	1.68	20.9	1.68	20.9	1.68
8	Ditto: + 618 lb. $\text{Am}_2\text{SO}_4$	...	...	25.9	2.08	57.2	1.84	15.9	1.80	15.9	1.80	15.9	1.80
9	Ditto: + 275 lb. $\text{NaNO}_3$ §	...	...	16.6	1.70	56.1	1.59	21.6	1.58	21.6	1.58	21.6	1.58
10	412 lb. $\text{Am}_2\text{SO}_4$ : no minerals	...	...	12.0	1.80	47.0	1.69	24.7	—	24.7	—	24.7	—
11	Ditto: + 3½ cwt. superphosphate	...	...	8.9	1.97	56.9	1.58	19.0	—	19.0	—	19.0	—
12	Ditto: + 3½ cwt. superphosphate + 366 lb. $\text{Na}_2\text{SO}_4$	7.1	1.43	13.5	2.07	57.3	1.55	22.9	—	22.9	—	22.9	—
13	Ditto: + 3½ cwt. superphosphate + 200 lb. $\text{K}_2\text{SO}_4$	9.3	1.61	17.4	1.86	55.2	1.64	25.6	—	25.6	—	25.6	—
14	Ditto: + 3½ cwt. superphosphate + 280 lb. $\text{MgSO}_4$	8.6	1.52	16.3	1.84	58.6	1.60	23.4	—	23.4	—	23.4	—
16	Complete minerals + 550 lb. $\text{NaNO}_3$ §	7.5	1.63	18.1	2.06	56.1	1.80	26.3	—	26.3	—	26.3	—

\* Taken from *Rothamsted Annual Reports*, 1926-9.

† In 1926 of flour only; in 1927-9 of wheat.

‡ Complete mineral manure = 3½ cwt. superphosphate, 200 lb. sulphate of potash, 100 lb. sulphate of soda, 100 lb. sulphate of magnesia.

§ Sulphate of ammonia is applied as to one-third in autumn and two-thirds in spring. Nitrate of soda is all given in spring, there being two applications at an interval of a month on Plot 16.

|| Independent millings: flour yields, 63.4 and 65.7 per cent. respectively.

¶ Independent millings: flour yields, 60.5 and 66.4 per cent. respectively.

in none of the crops was unsoundness of grain a factor in the poor quality observed in many of the flours.

Before proceeding to give a brief account of the broad features and relative behaviour in the bakehouse of each year's crops, it is necessary to point out the importance of adequate gas production in doughs during fermentation, and especially during the final, critical, proving period, *i.e.* the period when the loaves have been moulded and are waiting to go into the oven. The importance of this gas production as a factor in producing large loaves was first pointed out by T. B. Wood(9) in 1907. The later work of A. E. Humphries(5, 6, 7) and his collaborators showed that the quantity of gas given off in final proof does not determine the size of the loaf; it is rather a necessary condition for the production of a large loaf. It is obvious that a large loaf cannot result if gas production during proof is inadequate. Sufficient gas must be produced; then, if the other contributing factors are present (*e.g.* satisfactory gluten characters), a loaf of large volume may be made.

Table II. *Nitrogen contents and maltose figures of the Broadbalk flours, 1926, 1927, 1928 and 1929 crops.*

Plot	1926 crop	1927 crop		1928 crop		1929 crop	
	N %	N %	Maltose %	N %	Maltose %	N %	Maltose %
2 A	—	1.67	1.56	1.63	1.4	—	—
2 B	1.67	1.74	1.36	1.75	1.4	1.55	1.75
3	1.29	1.42	1.10	1.42	1.4	1.31	1.51
5	1.28	1.44	0.89	1.50	1.3	—	—
6	1.33	1.41	1.15	1.43	1.3	1.34	1.54
7	—	1.69	1.55	1.51	1.6	1.44	1.68
8	1.69	1.76	1.37	1.65	1.5	1.59	1.80
9	1.48	1.45	1.10	1.44	1.2	1.34	1.58
10	1.46	1.65	0.85	1.46	1.1	—	—
11	1.39	1.70	1.37	1.33	1.3	—	—
12	1.43	1.59	1.12	1.38	1.2	—	—
13	1.61	1.62	1.38	1.44	1.0	—	—
14	1.62	1.68	1.33	1.37	1.4	—	—
16	1.63	1.71	1.41	1.62	1.3	1.37	1.70

The power to gas freely and for a long period is a varietal characteristic in the sense that when grown under comparable conditions some varieties of wheat are always better gassers than others(5c). A given variety, however, may vary widely in gassing power from season to season, or according to environment during growth.

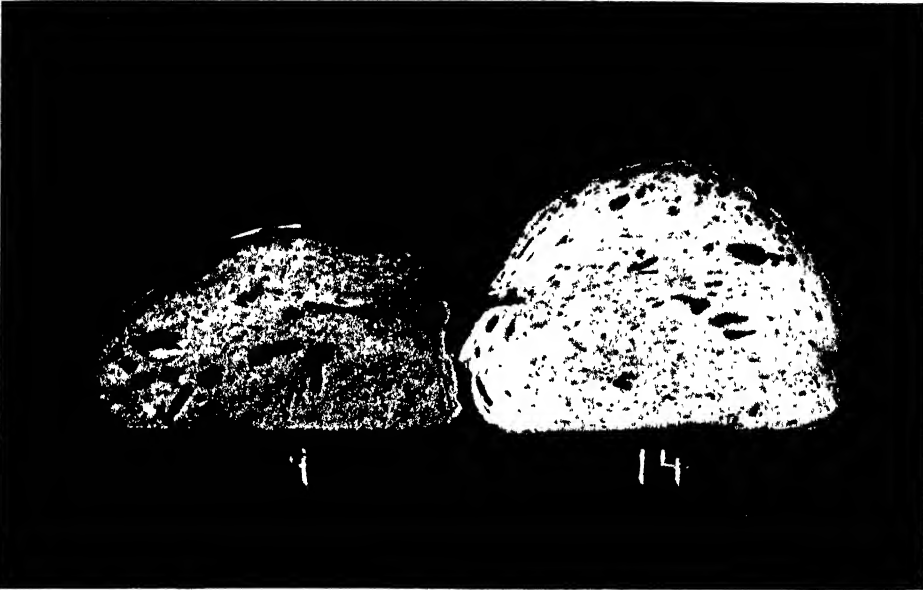
The importance of the point lies in the fact that unless adequate gas production can be ensured faulty conclusions may be drawn from baking tests: thus a flour of good gluten quality may behave in the bakehouse

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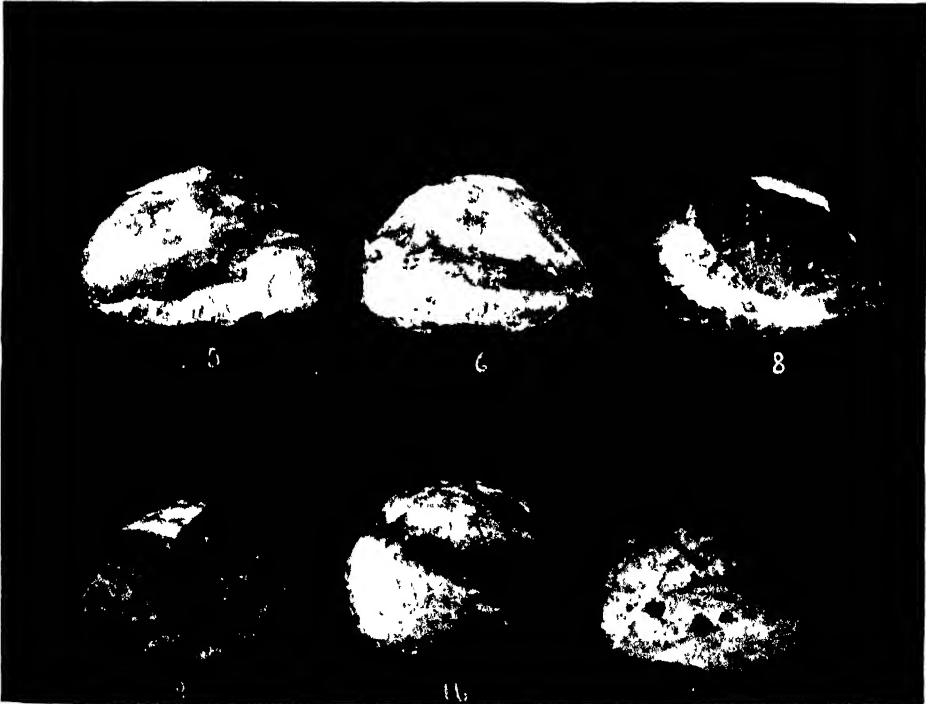
as badly as one of poor quality if gas production is insufficient. Fortunately, Humphries has shown that gas production can usually be increased sufficiently by the addition of small quantities (*e.g.* 0.1 per cent.) of highly diastatic malt extract with or without the further addition of acid ammonium phosphate (0.05 per cent.). In the baking tests to be described the usual baking process adopted was a fermentation of  $3\frac{1}{4}$  hours (including final proof) with 2 per cent. yeast and a dough temperature of 80° F. In some cases 8 hours' fermentation with 0.5 per cent. yeast was employed. With these processes it is important that sufficient gas be produced to inflate properly the dough (besides overcoming the considerable leakage that occurs from all doughs) in the last  $\frac{3}{4}$  hour (*i.e.* between  $2\frac{1}{2}$  and  $3\frac{1}{4}$  hours) when 2 per cent. yeast is used, and in the last hour (*i.e.* the eighth hour) when only 0.5 per cent. of yeast is employed. Gas production figures for four seasons' Broadbalk wheats were determined, but need not be given here. Gas production was maintained at a high and satisfactory level for 4 to 5 hours with 2 per cent. yeast, and for 8 hours with 0.5 per cent. yeast in the wheats of 1927, 1928 and 1929. The 1926 crop was definitely less satisfactory in this respect. With 2 per cent. yeast gassing was good for the first 3 hours, but a sharp fall occurred in the fourth hour. Gas production, therefore, in the bakehouse was probably satisfactory during the whole  $3\frac{1}{4}$  hours, and this is supported by the baking results given in Table III; although the two best loaves came from two of the three best gassers, the worst gassers did not produce the worst loaves. Broadly speaking, the baking results for the 1926 crop are probably comparable *inter se*. With the later crops malt extract and ammonium phosphate were used when necessary; the results for the three years 1927, 1928 and 1929 are therefore strictly comparable.

### THE 1926 CROP.

The wheats were of fairly high moisture content when received, and the mill feeds had moisture contents ranging from 17.1 to 18.4 per cent. With one or two exceptions the extractions or flour yields were very similar but low. This is because the work on this crop was carried out before our milling technique was modified in the direction of getting a better "finish" on the offals. In any case moderate differences in extraction in no way invalidate comparison between the plots. In one case (Plot 11) two samples of the wheat were milled independently with a deliberate difference in extraction of 6 per cent. (11 (i) 60.5 per cent.; 11 (ii) 66.4 per cent.), but the baking results were very similar with both



A. Worst and best loaves from 1926 crop of Broadbalk wheats.



B. Typical loaves from 1926 crop of Broadbalk wheats. Compare the torn inferior crusts with the unbroken and nicely checked crust of CCC, which is a good London flour made from a mixture of wheats.



samples<sup>1</sup>. It may be said, in general, that in a given year any differences in baking quality between flours milled from the respective plots which may be due to differences in the flour yields are so small as to be negligible in comparison with the relatively large differences due to variations in the quality of the wheats themselves.

The wheats of the 1926 crop were milled on various occasions between January 25 and March 28, 1927. In order to minimise any differences due to varying "age" of the samples the flours were not baked until May 31, 1927, when tinned loaves were made. With the exception of Plot 11 the flour extractions obtained ranged between 63 and 65·7 per cent. The flour from Plot 9 was very dirty: it was impossible to clean the wheat entirely satisfactorily on our small dry-cleaning plant before milling. It is not quite certain to what extent this circumstance influenced the baking results obtained on the 1926 crop from Plot 9.

The bread as a whole must be described as definitely inferior to that from average all-English non-Yeoman flour—unappetising in appearance, broken and very pale in crust with lifeless, coarse crumb of a greyish colour (cf. Plates III, A and B). Nevertheless, it was better than that obtained from Broadbalk crops of some later seasons.

Nos. 11 (i), 11 (ii) and 13 were outstandingly the best loaves. Their volume and oven spring were, indeed, good for all-English bread and their crumb was of fair all-round quality—its weakest features being spring and colour. The other extreme is represented by 2, 3, 5, 6 and 20, which were poor loaves with crumb which was "corky" and of poor spring. The general order of merit of the loaves is shown in Table III.

A second series of baking tests was carried out on August 3, 1927, when cob loaves were baked.

Each sample took liquor at the rate of 15 gallons per sack. The doughs as a whole were of the claylike, "lifeless" type; 9 was worst: it was very tender and sticky, "short" and claylike. The others showed a slight graduation in the order given in Table III, 12, 13 and 20 being best. These were still poor doughs, being short and claylike, but they were much tougher and more elastic than 9.

On the tray during the final proof for the oven considerable differences in stability were observed; 14 and 16 were bold, having very good stability; 11, 12 and 13 had good stability; whilst 9, 3 and 10 were poor. The others had very fair stability.

Of the cob loaves baked on August 3, 1927, 9 had deteriorated badly.

<sup>1</sup> This difference in extraction, moreover, was effected partly by more thorough cleaning of the bran and not only by heavier treatment of reduction stocks.

Table III. *Showing order of merit of the Broadbalk wheats in the various baking tests on each crop with respect to dough and loaf qualities.*

Crop	Date when baked			General character	Order in decreasing merit of the various plots
1926	31. v. 27	...	...	—	11 : 13 : 8 : 16 : 12 : 10, 14, 9 : 2 : 3, 5, 6, 20
	4. viii. 27	...	...	Claylike and short	12, 13, 20 : 14, 16 : 6, 8 : 10, 11 : 3, 5 : 2 : 9
		...	...	—	14 : 11   5 : 12 : 16   8 : 3 : 10 : 13 : 6, 20 : 2   9
1927	1. viii. 28*	...	...	Claylike	2 : 6, 7 : 8 : 3, 5   9, 10, 11, 12, 13, 14, 16
		...	...	Superior to 1926	2 : 6, 7 : 3 : 8 : 5, 10, 11, 14 : 12, 13, 16
	16. viii. 28†	...	...	Claylike	2 : 6, 7 : 8 : 3, 5   9, 10, 11, 12, 13, 14, 16
		...	...	—	2, 6 : 7 : 3, 5, 8 : 12   9, 10, 14 : 11, 13, 16
	April, 1929‡	...	...	Tough and short	7, 8 : 6, 9, 16 : 2, 5 : 3 : 11, 14 : 12, 13 : 10
1928		...	...	—	7, 8, 2, 11 : 6, 12   16, 14 : 9, 13, 5 : 3, 10
	April and July, 1929	...	...	Of different character from previous crops—tender, elastic, poor stability	6 : 3, 2, 5   16 : 8 : 13, 14 : 10, 12 : 11   7, 9
		...	...	Very poor	6 : 5 : 3, 8, 2 : 16, 10, 11, 12, 13, 14   7, 9
	Blended with No. 1 N. Manitoba flour:	...	...	—	All alike except 7 and 9 which were poor
	January, 1930	...	...	—	6, 8 : 10 : 2 : 3 : 11 : 5, 12, 13, 16   9 : 7
1929		...	...	—	As in January
	July, 1930	...	...	—	3, 6, 8 : 2, 13, 16, 5, 11, 12 : 10   9 : 7
	(Baked alone) 2. vii. 30	...	...	Fairly good, tender, elastic	16 : 9 : 2 : 3, 7, 8 : 6
		...	...	Superior to 1928	16 : 9 : 7 : 6, 3   8 : 2
	Blended with No. 1 N. Manitoba flour:	...	...	—	8§ : 2§, 7, 9, 16 : 3§, 6
	3. vii. 30	...	...	—	16, 9 : 7, 6 : 3   2 : 8

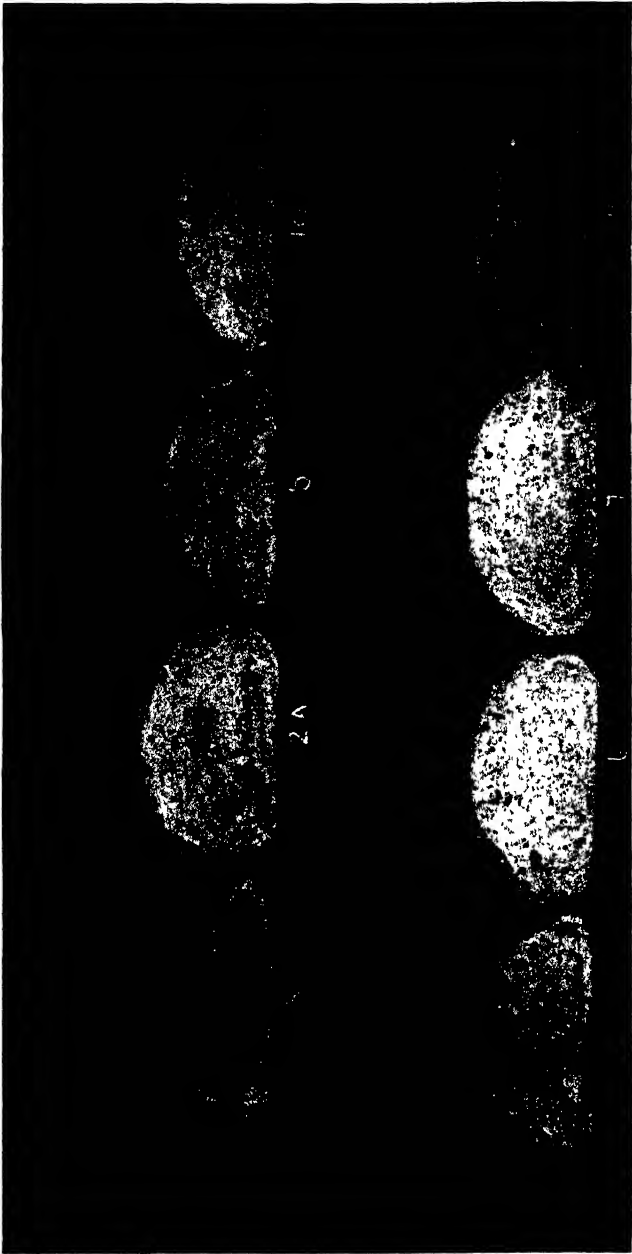
\* 0.5 per cent. yeast and 8 hours' fermentation.  
† 2 per cent. yeast and 3½ hours' fermentation.

† 1.25 per cent. yeast and 3 hours' fermentation.  
§ Poorest stability.

N.B. A vertical line in the table between plots implies a marked difference, a colon a less marked difference, whilst a comma implies no difference in quality between the plots so separated.







Cob loaves made from Broadbalk flours, 1927 crop. Plots 11, 12, 13, 14 and 16  
(not shown in this plate) were alike but inferior to 10.

The general order of merit of the loaves is assessed in Table III, from which it will be seen that whilst certain samples have markedly deteriorated, *e.g.* 9 and 13, others have improved, notably 14 and 5—at least relatively. The outstanding instance of this latter tendency is 14, which is now the best all-round loaf.

#### THE 1927 CROP.

The arrival of the 1927 crop, in February, 1928, followed a bad harvest, and the wheats as received had moisture contents ranging from 18 to 21 per cent. This made milling difficult, and accordingly in many cases the samples were partly air-dried at room temperature prior to milling. As good a finish on the offals as possible was obtained, but extractions varied between 65 and 72 per cent. The milling was carried out on various occasions between the middle of February and the end of July, 1928.

The samples were baked on August 1, 1928, using  $\frac{1}{2}$  per cent. yeast and 8 hours' fermentation, and on August 16 using  $1\frac{1}{4}$  per cent. yeast and 3 hours' fermentation. In both cases cob doughs were made and each was given liquor at the rate of 14 gallons per sack.

Throughout both baking trials corresponding doughs behaved alike. There was considerable variation in quality, but all the doughs were of the lifeless claylike type. Nos. 2, 6, 7 and 8 were easily best and handled satisfactorily. They were tough but "short" and had good stability. The remaining doughs were much poorer and handled very lifelessly. They are grouped according to general quality in Table III.

The bread varied widely in quality. The best loaves were excellent for all-English. Plate IV shows a group which includes the best loaves baked on August 1. Plot 2 gave the best all-round loaf. For all-English bread it had excellent volume and oven spring and good all-round crumb quality. Its crumb, in particular, lacked that "corkiness" characteristic of much all-English bread. It had good spring and a soft smooth texture.

The worst loaves were 12, 13 and 16 (not shown in photograph), which were much alike and rather poorer than 10, 11 and 14, which were also much alike and rather poorer than 5. Nos. 12, 13 and 16 were flat unappetising loaves of very poor, close-grained, cheesy crumb of bad colour.

With one or two exceptions the loaves baked on the shorter system (3 hours with  $1\frac{1}{4}$  per cent. yeast) tallied with those on the longer system. The general order of merit is shown in Table III for both baking tests. It will be noticed that 5 and 12 responded better to the short system than to the long.

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The 1927 crop was baked again in April, 1929. Cob loaves again were made, the absorption being still 14 gallons. The doughs were now very tough and "short" compared with those of the previous year's bake. Plots 7 and 8 were now best in point of all-round dough quality. They were of good body and tough, but very "short" and claylike. Plots 9 and 16 had improved relatively and were now only inferior to 7 and 8. The doughs were all much of the same type, and there were none which were outstandingly inferior to others.

Many of the samples still made satisfactory bread. Plots 2, 7, 8 and 11 had good oven spring and volume, and crumb which was very close in grain but of satisfactory spring, pile and texture. Plots 6 and 12 were only slightly inferior to these, but the others were a considerable way behind, especially in crumb quality.

The order of quality both of doughs and of loaves is given in Table III.

It will be seen that 11 had markedly improved, whilst 3 had markedly deteriorated.

### THE 1928 CROP.

The 1928 crop was the first outcome of the change in procedure of cultivation described in the introduction, and the improved conditions were at once obvious in the appearance of the wheats. The samples were very much cleaner, and the grain was greatly improved in appearance and milling character.

When received in December, 1928, the samples had moisture contents ranging from 17 to 21 per cent. To overcome as much as possible the difficulty of varying age in the flours owing to the considerable time over which the milling of the various samples had to be spread, the samples were milled and baked in two groups. Comparison was effected through the inclusion of Plot 3 in both groups.

Plots 3, 6, 7, 8, 9 and 16 were milled during the first fortnight in March, 1929, and a baking test carried out in April. Plots 2 A, 2 B and 3 (a second sample), 5, 10, 11, 12, 13 and 14 were milled between the middle of April and the end of May, and baked in July, 1929.

When milled the samples of wheat, which had been stored in bags in a cool dry place, had dried appreciably and on the whole they milled very well, though the moisture contents of the mill feeds ranged between 13 and 18 per cent. The extractions obtained ranged between 70 and 74 per cent.

The change for the better in condition of the wheats was reflected in

the better appearance of the flours and, in turn, in a vastly improved crumb colour in the bread. On the whole, the crumbs had a pleasing creamy white colour—in marked contrast to the lifeless greyish crumb of previous years. These features were accompanied by a radical change in type of the doughs as a whole and, unexpectedly, by a marked change for the worse in general bread quality. Instead of being claylike and lifeless but stable, the doughs were for the most part tender and extensible with poor stability. As with the 1927 crop, absorption was 14 gallons per sack for cob doughs. Plot 6 gave the best dough. It had good body, and was fairly tough and extensible with fairly good spring. Plots 3, 2 and 5 were also satisfactory, but the remaining doughs tended to be excessively tender. Plots 7 and 9 were outstandingly the worst; they were scarcely better than batters, were almost impossible to handle and had to be scaled into tins—it was impossible to bake cob loaves from them. They were obviously unable to carry as much water as the other samples.

All the loaves were more or less flat, lacking boldness—a feature which reflected the inferior stability of the doughs. Altogether the bread was so poor that it was difficult to make satisfactory comparison. However, a guide to the relative value of the plots, both in doughs and bread, is given uniformly with those of other years in Table III.

Plot 6 gave probably the best loaf. It had only fair oven spring and volume, with crumb which was open but even in grain, of only fair spring and rather coarse texture with a creamy white colour. The loaves from Plots 2 A and 2 B were very much alike, and of inferior crumb spring to 6; 7 and 9 were distinctly the worst: these two flours had probably the poorest baking quality within our experience.

An alternative and valuable method of comparison is available even in the case of very poor samples such as these. It consists in blending each sample to the same extent with “strong” flour, such as that from No. 1 Manitoba wheat.

In the present instance 50–50 blends of the flours from the various plots, respectively, with a sample of No. 1 Manitoba straight-run flour were baked on January 16, 1930. Cob loaves were baked, using 2 per cent. yeast and a fermentation time of 4 hours, and 0.1 per cent. malt extract and 0.05 per cent. ammonium phosphate was incorporated into each dough in order to avoid any complications which might possibly arise through inadequate gas production.

Each dough took liquor at the rate of 16 gallons per sack. With the exception of 7 and 9 there were no marked differences between the

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handling properties of the blends, all of which had good body and were fairly tough and elastic, and handled tightly with good spring. Plots 7 and 9, however, tended to become unduly tender as fermentation proceeded. They moulded distinctly more tenderly than the others (7 being the poorer of the two) and, alone of the blends, showed inferior stability in the final proof.

Plots 6 and 8 gave the best loaves of all the blends. They had very good oven spring and volume, slightly torn but otherwise good crusts and good outside appearance, very close and even grain of crumb, which was of good spring, pile and texture, with a good pale grey-white colour. Plots 7 and 9 were easily the poorest loaves (7 being poorest of all); 7 had very poor oven spring: it was a flat loaf, with a reddish, torn crust, and crumb (of the "English" type) of a fine honeycomb grain with poor spring and a rather poor though creamy colour.

The plots are arranged in order, according to general quality shown in this baking test, in Table III.

These flours were again blended and baked in exactly the same way on July 4, 1930. Their behaviour in the dough was similar to that during the first series of blending tests. The loaves also on the whole were very similar to those of the earlier tests. Again 7 and 9 were markedly the poorest (7 being the poorer). Plot 3 was now, however, quite as good as 6 and 8. These three loaves were the best and were like the best loaves of the January bake. Differences between the remaining loaves were not marked. The general order is shown in Table III.

### THE 1929 CROP.

The arrival of the 1929 crop in February, 1930, followed a good harvest, and the wheats were in excellent condition. As received, they had moisture contents between 15 and 16 per cent. They milled well, extractions varying between 70 and 72·7 per cent.

The range of plots dealt with was restricted this year to the following seven: 2, 3, 6, 7, 8, 9 and 16, which were of greatest interest from the point of view of nitrogenous manuring. The samples were milled during March and April, 1930, and baked on July 2, 1930.

Each sample was given liquor at the rate of 16 gallons per sack, which, allowing for the fact that tinned doughs were made, indicated a distinctly higher absorption than that of previous years. 3½ hours' fermentation (with 2 per cent. yeast and 1½ per cent. salt) was used and 0·1 per cent. malt extract and 0·05 per cent. ammonium phosphate was added to each dough.

The doughs as a whole were fairly good in handling properties, and were of the same type as the 1928 crop—more or less tender and extensible. Plot 16 gave distinctly the best dough; it was fairly tough and of fairly good spring and elasticity. Plot 6 gave the tenderest dough.

The bread was better than that of the 1928 crop. The best loaf was 16. It had very good oven spring and volume, good outside appearance and crust with a good face. Its crumb was fairly close and even in grain, of good spring, pile and texture, and of a pale grey-white colour.

The loaves from Plots 2 and 8 were much poorer than the others. They had open coarse grain of crumb, and were of inferior spring, pile and texture.

As in the case of the 1928 crop, a test was also made in which each flour was blended with a sample of No. 1 Manitoba flour. The proportions used this time were 40 per cent. Broadbalk flour with 60 per cent. No. 1 Manitoba flour. Cob doughs were made and the procedure was the same as with the 1928 crop. All the blends made doughs of good body, fairly tough with good spring and extensibility, but all tended to fall off in body rather unduly as fermentation proceeded. Plots 3 and 6 became appreciably more tender, whilst 8 remained tougher than the others. On the tray at final proof 8, 3 and 2, in that order, showed the greatest flow.

Plots 16 and 9 gave the best loaves and were but little inferior to the all-Manitoba control which was a bold loaf of excellent oven spring and volume, and very good outside appearance and crust: it had good soft crumb, fairly close and even grain, and very good spring with a pale grey-white colour.

Plots 8 and 2 were much poorer than the others. Their crumb was open and coarse in grain, and of only fair spring, pile and texture, with a dull grey-white colour.

Table III shows the behaviour of the 1929 plots with respect to one another in dough and loaf, both when baked alone and when blended with the Manitoba flour.

#### DISCUSSION OF RESULTS.

Inspection of Table III, which shows the relative order of merit of plots of the respective crops, as revealed in early and in later baking tests, shows that no definite trend of improvement or of deterioration on storage can be assigned to any particular plot. For example, Plot 3 deteriorated markedly during storage in 1927, but improved relatively

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to the other plots in 1928. Plot 11 tended to fall off in 1926, but showed relative improvement in 1927.

Humphries(8), in the trials referred to, found that of the 1903 crop Plots 10 and 11 improved markedly after some months' storage, whereas Plots 2 and 3 did not significantly change. He found a general and fairly marked improvement on storage amongst members of the 1904 crop.

Table IV has been prepared for purposes of general comparison from the preceding table. It represents an attempt to "average" the results of the earliest baking tests for each crop so as to show the general order of merit, with respect to dough and to loaf quality, of the respective plots, year by year.

Table IV. *General order of merit of the Broadbalk wheats with respect to dough and loaf qualities of several consecutive crops "averaged" as far as possible for each crop.*

1926 crop	1927 crop		1928 crop		1929 crop	
Loaves: generally poor	Doughs	Loaves: superior to those of 1926	Doughs	Loaves: very poor	Doughs	Loaves: superior to 1928
11	2	2, 6	6	6	16, 9	16
13	6, 7	7	3, 2, 5	8	7, 8	9
8	8	3, 8, 5	16	2, 3	2	7
16	3, 5	12	8	5, 10	3	6
12	9, 10, 11, 12	9, 10, 14	13, 14	11	6	3
10, 14, 9	13, 14, 16	11, 13, 16	10, 12	12, 13, 14, 16	—	8, 2
2	—	—	11	9	—	—
3, 5, 6, 20	—	—	7, 9	7	—	—

N.B. A horizontal line in the table between plots implies a marked drop in quality between the plots so separated.

It will be seen that it is not possible to draw any definite conclusions as to the effect of manurial treatment upon baking quality from the results for these four years, let alone from those for any one season only. The order of quality is essentially different every year. The best plot one year is the poorest another year and so on. There is no plot which may safely be said to maintain either a very high relative position or a very low one, and there is no question of any one plot retaining an unaltered quality whilst the others fluctuate.

Humphries(8) found that of the 1903 crop, which on the whole was poor, Plot 2 was the worst. He describes it as the worst flour he had ever seen. It evidently therefore rivalled Plots 7 and 9 of the 1929 crop, but it will be noticed that Plot 2 of the 1927 crop was relatively of excellent



quality. Humphries claims as his most important result the superiority of the wheat from the unmanured plot in both 1903 and 1904. It is evident, however, from Table IV that Plot 3, whilst not generally by any means the poorest plot, does not maintain a high position.

Plots 7, 10 and 11 were poor both in 1903 and 1904, and especially in the earlier year when climatic conditions were bad. Here again the harvests of a later decade bring no confirmation.

Without doubt the factors operating on wheat quality in the Broadbalk Field are so complex that adequate analysis may be based only on observations extending over a long period. It seems probable that varying climatic conditions play a great part and complicate any attempt to influence wheat quality directly by the simple application of fertilisers.

Although there thus seems no possibility, as far as the work already done on the Broadbalk wheats is concerned, of establishing the definite superiority of one type or dosage of manure as against another from the point of view of baking quality, it is perhaps worth while to make the following observations:

Apparently Plots 10, 11, 12, 13 and 14 associate together on the whole so that the presence or absence of given basic constituents, or of phosphates, in the fertiliser appears to have no discernible effect upon quality of the grain.

Plots 6 and 8 on the one hand and Plots 9 and 16 on the other tend to run together. When 16 and 9 are high in the order of relative merit then 6 and 8 are low and *vice versa*. Moreover, when 16 and 8 are good (or bad) then 9 and 6 also tend to be good (or bad). That is, when the nitrate plots are good the ammonia plots are poor and *vice versa*. This does not apply to the 1926 crop, however, when dose rather than type of manure appeared the more important, and 6 and 9 were both of low quality, and 8 and 16 both relatively higher in quality. When the ammonia plots are better and the nitrate poorer, the unmanured is up in quality and *vice versa*; except in 1926 when dose was important, and 3, the unmanured, was down in quality. No. 2, the dunged plot, tends to associate with 6, the plot with the smallest dose of ammonia.

It would be premature to speculate as to the causes which produce these apparent associations, and it will be necessary to collect more data before attempting to establish a differentiation between the action of nitrate and of ammoniacal fertilisers on the growing crop. One naturally has climatic influences most in mind and it is suggestive that the 1929 crop (when the apparent nitrate ammonia see-saw had turned in favour of the nitrate) followed an exceptionally warm dry summer. It is just possible

that under one set of climatic conditions the one type of manure tends to earlier assimilation or more rapid maturity than the other, with consequent differences in colloidal characteristics of the protein gel, but without affecting the total amount of nitrogenous material in the ripened grain.

It must be emphasised that the foregoing observations with respect to relative qualities of the wheats in no way imply corresponding differences in protein content. A comparison of Table II with Table IV shows that there is no correlation between the protein content of the Broadbalk flours and their baking quality. It is well known that it is necessary to distinguish between amount of protein and quality of protein in connection with "quality" in wheat.

In spite of the obscurity of the whole problem suggested by the observations recorded above, there are several lines of attack which readily suggest themselves. Two of these we have had the opportunity of exploring in a preliminary manner.

In the first place, it is necessary to explain that with many flours whose protein is relatively high in amount but of such physico-chemical properties that the flour is "weak," in the sense of producing soft doughs of low water absorption and poor stability, it is possible to influence baking quality favourably by appropriate physical treatment of the flour. Various methods of carrying out this treatment are known, two of which have been developed by the present writers<sup>(4)</sup> and patented in Great Britain and several other countries. We have found that the best method of carrying out the treatment on a small scale is to expose the flour in thin layers on trays to the action of a moving hot air belt of relative humidity above 60 per cent. and at a temperature of 160° F. for a period of about 15 minutes.

In 1929 portions of the flour from Plots 3 and 8 of the 1928 crop were heat treated in this way. The treated samples were baked together with the untreated.

As a result of the treatment both samples made tougher doughs of unimpaired elasticity and improved stability and handling quality generally. The bread from both untreated samples was very poor—as indeed had been found to be the case in the earlier bake on the 1928 crop as summarised in Table III. It was of only fair oven spring and volume, with open grained coarse crumb of only fair pile and texture and poor spring. Plot 3 was distinctly improved by the treatment, giving a loaf of fairly good oven spring, close and even grain of crumb which was of good pile and slightly better spring and colour than the untreated.

Plot 8 was very markedly improved by the treatment. The treated loaf was bold, of good oven spring and volume with crumb close and even in grain, of good spring, pile and texture, and of much better colour than the untreated.

The 1928 crop as a whole was of inferior baking quality. The doughs were tender and the bread very poor. It may be assumed, therefore, that the gluten was in such a physico-chemical condition that it implied "weakness" *per se*, but that it was capable of modification and improvement by suitable heat treatment. This being so, the flour of higher actual content of protein (Plot 8) would be expected to show the greater improvement.

In 1930 a portion of each sample of the 1929 crop was heat-treated and baked alongside the untreated flour.

Plot 2 showed marked all-round improvement as a result of the treatment. The loaf was bolder with better bloom of crust, the crumb was closer in grain and paler in colour. Plot 8 was appreciably improved though rather less markedly so than 2. Plots 3, 6 and 9 were slightly improved, whilst 7 and 16 were practically unaffected.

In the case of Plot 2 of the 1929 crop a further test was carried out in which 30 per cent. of the untreated flour and 40 per cent. of the treated were blended with 70 per cent. and with 60 per cent. respectively of untreated No. 1 Manitoba flour. Notwithstanding the additional amount of English flour carried, the treated blend gave a dough of markedly better body, and a better all-round cob loaf, than the untreated blend.

These results offer a definite suggestion that where the protein content has been increased as a result of manurial treatment, but is "weak" from the baking standpoint, it can be improved by certain physical treatment, whereas if it has been increased in amount and is satisfactory from the baking standpoint, or when low has not been increased in amount, the flour may not respond to physical treatment. The problem may be stated as follows: What are the conditions accompanying the production of increased protein content as a result of a certain definite manurial treatment which in one season confer colloidal properties upon the protein such as are associated with "weakness" and which can be modified by physical means in the direction of increased "strength," and which in another season form the colloid so that it is of satisfactory baking quality and less susceptible to improvement by physical means?

The other avenue of exploration is indicated by work carried out in the United States by J. Davidson (1, 2, 3) and collaborators. These workers studied the effect of the application of nitrogenous manures at various

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stages during the growth of the wheat plant on the crop yield and nitrogen content of the grain. In general they found:

(1) The effectiveness of nitrates in increasing yields decreases progressively as the time of application approaches heading time.

(2) The effectiveness of nitrates in increasing protein content of the grain varies in the inverse sense to their effectiveness in increasing yield as far as heading time.

(3) After heading time (*i.e.* in the milk stage) the effect on yield was nil, and the effect on protein content was less than at heading time.

During 1928 and 1929 some preliminary trials were carried out at Rothamsted to test these conclusions. Four varieties of wheat were grown, viz. Yeoman II, Swedish Iron, Squarehead's Master and Million III. These were given respectively the following treatments:

(1) No top-dressing.

(2) No top-dressing.

(3) Sulphate of ammonia, 1 cwt. per acre applied early, *i.e.* at tillering.

(4) Muriate of ammonia, 1 cwt. per acre applied early, *i.e.* at tillering.

(5) Sulphate of ammonia, 1 cwt. per acre applied late, *i.e.* 8 weeks later.

(6) Muriate of ammonia, 1 cwt. per acre applied late, *i.e.* 8 weeks later.

(7) Sulphate of ammonia, early and late (*i.e.* a double dressing).

(8) Muriate of ammonia, early and late (*i.e.* a double dressing).

In each case the treatment was carried out on triplicate plots, each of one-fortieth acre: 96 plots in all.

Nitrogen determinations were carried out on each of the ninety-six samples of dressed grain received in each of the years 1928 and 1929. The results may be given shortly here: there was no significant increase in nitrogen content due to the manurial treatment in any instance.

Subsequently, certain of the triplicate samples of the 1929 crop were bulked so that milling and baking tests could be carried out. Four samples of straight-run flour were obtained from the Yeoman wheats, representing the following manurial treatments:

					Nitrogen content of the bulked sample of wheat
A 1.	Unmanured	...	...	...	1.47 %
A 2.	Sulphate of ammonia	early	...	...	1.49 %
A 3.	"	"	late	...	1.49 %
A 4.	"	"	early and late	...	1.43 %

A similar series of samples was obtained of the Squarehead's Master series:

					Nitrogen content of the bulked sample of wheat
B 1.	Unmanured	...	...	...	1.50 %
B 2.	Sulphate of ammonia	early	...	...	1.53 %
B 3.	"	"	late	...	1.52 %
B 4.	"	"	early and late	...	1.49 %

Extractions, *i.e.* flour yields, ranged between 71.5 and 73.3 per cent. of the wheat. The samples were milled during April and May, 1930, and baked during May and June.

Both series were baked on the multiple differential system developed in the writers' laboratories—in the first place alone, *i.e.* unblended, using different fermentation periods with 2 per cent. yeast; secondly, with 2 per cent. yeast and with a fixed fermentation period, but blended to the extent of 40 per cent. with 60 per cent. of each of two reference flours. These reference flours were straight-run commercial flours—a "strong" London flour and a relatively "weaker" north country flour respectively.

The Yeoman samples behaved alike in the dough. They made doughs of good body, which were claylike with fairly good stability but only fair spring; they tended to fall off unduly in body as fermentation proceeded. The bread, however, showed that the fermentation time of the samples, which was relatively long in all cases (*i.e.* 4 hours or more), lengthened slightly but progressively from A 1 to A 4, but that A 2 and A 3 gave distinctly better all-round loaves than A 1 and A 4. A 2 was slightly better than A 3; A 1 and A 4 were much alike. These results were substantially confirmed by those of the blending test.

The Squarehead's Master samples gave poor doughs and poor bread. The fermentation time was shorter than that of the Yeoman, but it was the same for all four samples: the best loaves in each case followed 3½ hours' fermentation. There was no difference between the doughs of the four samples, but 3 gave distinctly the best bread and 1 the poorest; 2 was slightly better than 4.

A portion of each sample of flour was now heat-treated on the same lines as the Broadbalk samples. With the Yeoman samples all were distinctly improved by the heat treatment except A 3 which was only improved slightly. The effect of the heat treatment on fermentation time was slightly but consistently to increase it—less with the unmanured sample than with the manured—though 3 was less affected than 2 and 4.

With the Squarehead's Master samples there was no noticeable

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lengthening of fermentation time due to heat treatment, but the optimum loaves of samples B 1 and B 2 were markedly improved by the heat treatment, whereas those of B 3 and B 4 were slightly deteriorated.

Although it is not possible from the work described above to draw any definite conclusions as to the effect of the manurial treatment of wheat upon the baking quality of the flour, the work has revealed the extreme complexity of the problem and of the various factors, climatic and other, that appear to influence quality.

Moreover, two promising lines of investigation into the problem have been opened up. There is some evidence that the protein content of wheat may be materially increased by certain manurial treatment, although the precise nature and conditions of such treatment have so far eluded discovery. There is also evidence that the improving effect of certain types of heat treatment on "weak" English flours may be greater the greater the protein content of the flour. Further, the resulting improvement is more pronounced when the improved flour is blended with a "stronger" flour than when it is baked alone. If these provisional conclusions can be established as fact, they would have considerable economic importance in connection with wheat growing in this country. If the area under wheat is not to diminish still further, still more if the area is to be increased, it is essential that the wheat be grown, ground and consumed locally in order to avoid the heavy transport charges characteristic of this country. To bring this about it must be made possible for the country miller to use a greater proportion of English wheat in his grist. This can be done if the quality of the wheat can be improved in the two-fold manner indicated.

### SUMMARY.

In each of the four seasons 1926 to 1929 samples of certain of the Broadbalk wheats were milled, and the straight-run flours tested in the bakehouse.

The results given in the text show that the nitrogen contents of the wheats were increased consistently only by the heaviest nitrogenous treatments. The baking tests, however, showed that increased protein content was not necessarily accompanied by improved baking quality. In none of the crops was unsoundness of grain or inadequate gas production during fermentation a factor in the poor quality observed in many of the flours.

The baking results are summarised in Tables III and IV. The order

of the quality of the various plots was essentially different every year. No plot maintained either a high or a low relative position, and there was no question of any one plot retaining an unaltered quality whilst others fluctuated. No definite trend in degree of improvement or deterioration during storage of flour could be assigned to any particular plot or to the plots as a whole.

Evidently the factors operating on wheat quality in the Broadbalk field are so complex that adequate analysis may be based only on very extended observations, but certain tentative conclusions may be drawn from the results discussed. The presence or absence of given basic constituents, or of phosphates, in the fertiliser had no discernible effect upon the quality of the grain. Further, in years when nitrate fertiliser produced good results, the ammonium fertiliser gave poor effects and *vice versa*. The unmanured plot tended to be of better quality when the ammonia plots were better and the nitrate poorer, and *vice versa*. The dunged plot tended to associate with the plot receiving the lightest ammonia dressing.

It is possible that climatic influences determine the effect of one type of manure as opposed to another in producing favourable or unfavourable colloidal characteristics in the protein gel of the ripened grain without affecting the total amount of nitrogenous material present.

The Broadbalk flours appear to vary in the extent of their response to a process of heat treatment, which, with many flours, is known to have the effect of modifying the physico-chemical condition of the gluten favourably (*i.e.* in the direction of improved baking quality). Experiment tended to show that where protein content has been increased as a result of manurial treatment, but the flour is "weak" from the baking standpoint, improvement follows the physical treatment, whereas if the protein has been increased in amount and is satisfactory in quality, or when low has not been increased in amount, the flour may not respond to physical treatment. In the case of one flour where improvement following heat treatment was marked, the effect of the treatment was even more marked when baking tests were carried out on blends of the untreated and treated flour with an untreated "strong" No. 1 N. Manitoba flour.

Flours were examined also from wheats grown at Rothamsted during 1929 to test the effect of the application of nitrogenous manure (in the form of ammonium salts) at an early and at a late stage of plant development respectively. The growing trials were comprehensively planned but no significant increase in nitrogen content due to manurial treatment was found in any instance. The doses, however, were less than those used on

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the Broadbalk plots and were perhaps more in accord with commercial practice. The results of the baking quality comparisons were not conclusive, though with both Yeoman and Squarehead's Master certain of the manured samples had appreciably better quality than the unmanured.

We desire to express our thanks to our colleagues, Mr R. H. Carter and Dr P. Halton, for the large amount of work involved in providing us with the analytical and gas production data. Our thanks are due in a special degree to Sir John Russell, F.R.S., for so readily giving us the wheats and for modifying a Rothamsted field experiment in 1928 and in 1929 to enable us to carry out the preliminary study of the effect of nitrogenous manuring on protein content described in the latter part of the paper. The baking tests were carried out by Mr W. E. Spencer, baker to the Research Association, whose skilful help we wish to acknowledge.

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# THE EFFECTS OF SUMMER GREEN MANURES ON THE AMMONIA AND NITRATE CONTENTS OF SOILS CROPPED FOR WINTER WHEAT.

## AN EXAMINATION OF THE WOBURN GREEN MANURE PLOTS<sup>1</sup>.

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(With Three Text-figures.)

THE purpose of the Woburn experiments on green manuring was defined by Dr J. A. Voelcker in 1892 in the following words<sup>1</sup>: "Following up the recently ascertained truths respecting the assimilation of the free nitrogen of the air by the leguminous plants, it was sought to ascertain whether by growing green crops of a leguminous nature and ploughing them in as a preparation for a corn crop, a better corn crop will result through the enrichment of soil by accumulated nitrogen than where a non-leguminous crop was grown." The results were surprising. After tares (vetches-*Vicia sativa*) the wheat crop was almost invariably poorer than after mustard (*Sinapis alba*) and, further, the yields on both sets of plots steadily declined. Summarising the results 35 years later, Dr Voelcker said in a contribution to a Rothamsted Conference on Green Manures(2): "Altogether it seems quite unaccountable that such miserable crops of wheat should follow the pursuit of what may ordinarily be good farming practice. It is quite evident that there must be some factor as yet unknown to us, which produces a result not only at variance with scientific deductions, but with practical experience generally, for without assuming some disturbing element of this nature, it is incomprehensible that liberal treatment such as these plots have received could result in the production of crops so meagre. Many have been the attempts I have made to find a possible explanation and many the suggestions put forward but none has so far been found to be tenable."

The Rothamsted Conference in 1927 revealed a number of other cases in which green manures, especially tares, had given disappointing results

<sup>1</sup> This paper is abridged from a portion of a "Thesis approved for the Degree of Doctor of Philosophy in the University of London."

in the following wheat crop though in no other case have observations been continued long enough to give such complete crop failures as at Woburn.

The work to be described in this and the following papers was undertaken to test the hypothesis that the production of relatively large amounts of nitrate during autumn and winter and their loss by drainage lead to a deficiency of available nitrogen at a critical period for wheat in the following summer. Systematic analyses of the soils of the Woburn green manure plots and small scale experiments with top dressings of sodium nitrate were carried out in the seasons 1928-9 and 1929-30, and are described here. The results of laboratory and pot-culture experiments with a fuller discussion of the hypothesis are given in a later paper(3).

#### LANSOME FIELD GREEN MANURE PLOTS.

In the original experiment in Lansome field a wheat and a green manure crop have been taken in alternate years with a few exceptions since 1892. Generally there were two green crops during the summer and both were ploughed in. The mean yields of wheat following green manures were 12·4 cwt. per acre after mustard and 8·6 cwt. per acre after tares. On two occasions when a second cereal crop was taken after the wheat there was no difference between the tares and mustard. Until 1917 there was a third plot with rape which gave similar results to the mustard plot. In 1917 the experiment was rearranged; the rape plot was abandoned and a new pair of mustard and tares plots and one with a summer fallow started. Mineral manures and lime applied to half of the plots gave slight increases in yield but did not alter the relative effects of the green manures. From 1917 to 1927 the wheat crops on each of these plots were miserably small; only two (1923 and 1925) were considered worth harvesting and these gave below 4 cwt. of grain per acre. The crop failure appeared complete until 1928-9, when the yield returned to a reasonable size (20·6 and 15·3 cwt. of grain per acre on the old tares and mustard plots respectively) and for the first time the tares gave a better crop than mustard.

#### STACKYARD FIELD GREEN MANURE PLOTS.

In 1911 a second series of plots was started in Stackyard field. The two summer crops of green manures were consumed on the plots by sheep which received cotton cake at the rate of 3 cwt. per acre. In 1921 the rotation was interrupted by taking a second wheat crop on half of the plots for one year so as to provide both wheat and green manure crops

each year. In 1923 one-half of each plot was limed. There are thus 8 plots in the experiment in sets of 4 cropped alternately with wheat and green manures.

At the commencement the land was known to be in poor condition, but the first wheat crop (1912, 10.1 and 9.8 cwt. grain per acre after tares and mustard) though poor has never been equalled. The falling off in yield proceeded more rapidly on the tares plots than on the mustard plots and was still further accelerated by liming (average yield for 12 crops 4.8 and 5.4 cwt. grain per acre after tares and mustard respectively on the unlimed portions).

It happens almost invariably that during the winter months the wheat after tares appears more advanced and healthier than that after mustard, and that both of them compare very favourably with other wheat in more normal rotations. About May the wheat after green manures falls behind other wheat and in the first few rotations the failure occurred earlier on the wheat after tares. On several occasions during the thirty years the dry weights and nitrogen contents of the green crops were estimated from samples in both fields and showed that the tares contained more nitrogen. Soil analyses showed that the tares plots contained more nitrogen than the mustard plots in both fields.

#### WHEAT AFTER GREEN MANURES 1928-9.

The wheat crops of 1928-9 in the two fields differed in one important particular. On Lansome field the customary two successive green manure crops were grown during the summer (tares from May 14 to July 13, and from July 14 to October 28, 1928; mustard from May 30 to July 13, and from July 27 to October 27, 1928) and wheat was sown almost immediately (November 1). On Stackyard field, however, there was only one crop of each green manure (tares, sown May 16, folding July 31 to August 8, 1928 and mustard sown May 31, folding July 23 to July 30, 1928). From the beginning of August until November 6 the plots were bare fallowed. During these fallow periods there were 51 days with rain and a total rainfall of 6.75 in., with 6 extra rainy days and 1.06 in. extra rainfall after the folding of the mustard and during that of the tares. This high and well-distributed rainfall provided opportunity for nitrification and leaching out of some of the nitrate formed in Stackyard field, whereas on Lansome field much of the nitrate formed during this period would be absorbed by the green crops and returned to the soil at the beginning of a dry period.

## SOIL ANALYSES ON WHEAT PLOTS, 1928-9.

Throughout the season 1928-9 soil samples were taken at frequent intervals from each of the 8 wheat plots in Stackyard field and the 5 wheat plots in Lansome field at depths of 0-6 in., 6-12 in. and 12-24 in., each sample being a composite one of 6-8 cores. With a minimum of delay the fresh soils were analysed for ammonia (in the first few samples by Matthew's aeration method and subsequently by the salt extraction method) and nitrate (by the phenoldisulphonic acid method). The salt treatments or extractions were always completed on the day of sampling.

The differences between the limed and unlimed series in Stackyard field and between the old and new series of plots in Lansome field were relatively small and the results for the corresponding pairs of plots have therefore been averaged in the following statements so as to reduce chance effects from soil irregularities in systematically arranged plots. The results are given in Fig. 1 for the first foot of soil only; Table I includes the averages for the second foot samples throughout the year. In Stackyard field it was possible to compare the permanent green manure-wheat plots with three other wheat plots under more usual rotations, viz. Series C, wheat in Norfolk rotation with either corn or cake fed to the sheep during the folding off of swedes, and a special strip of wheat immediately adjoining the mustard plots and following potatoes manured with dung. The average values for these three rotational wheat plots are also given in Table I.

During the winter and early spring the wheat after green manures stood out as by far the best and most forward wheat on the farm. Up to the beginning of May there was little difference between the Lansome and the Stackyard plots. This may be illustrated by the figures in Table I for the average nitrogen contents of the plants on May 9 (2.1 mg. and 2.5 mg. for Stackyard and Lansome).

From this time onwards the Stackyard wheat made very slow progress but the Lansome wheat grew vigorously. The difference between the two fields is well illustrated by the average nitrogen contents per plant on June 16 (4.3 mg. in Stackyard and 10.5 mg. in Lansome). In the five weeks' interval the nitrogen content of the Stackyard wheat had doubled, but that of the Lansome wheat had increased fourfold. This difference became steadily greater right up to harvest.

Fig. 1 also shows the monthly rainfall. A very wet October and a normally wet November were followed by an exceptionally dry and cold

spell in January, February, and March with a pronounced drought again in June, July, and August.

Table I.

	Stackyard field. Green manures eaten off Wheat after			Lansome field. Green manures ploughed in Wheat after		
	Tares	Mus- tard	(Rota- tion)	Tares	Mus- tard	(Fal- low)
Ammonia N in p.p.m. soil 0-12 in.						
Means of three dates of sampling						
Nov.-Dec. 1928	8.6*	9.4*	5.9*	—	—	—
Jan.-Feb. 1929	7.5†	5.3†	5.4†	7.8	5.7	—
Mar.-Apr. 1929	2.5	4.6	2.8	7.5	5.1	7.5
May-June 1929	1.2	1.1	1.2	1.8	2.3	2.3
Mean of all determinations: 0-12 in.	3.5	3.9	3.0	5.1	4.0	(4.2)
12-24 in.	3.3	3.8	2.9	3.6	5.1	—
Nitrate N in p.p.m. soil 0-12 in.						
Means of three dates of sampling						
Nov.-Dec. 1928	1.8	1.6	2.9	1.6	0.9	0.9
Jan.-Feb. 1929	0.9†	0.6†	1.0†	1.0	0.9	1.2
Mar.-Apr. 1929	1.7	0.9	1.4	2.0	1.7	1.8
May-June 1929	1.1	1.0	1.4	1.6	1.6	1.4
Mean of all determinations: 0-12 in.	1.3	1.0	1.3	1.6	1.2	1.3
12-24 in.	1.0	0.9	1.6	1.4	1.1	1.1
Mean N content per plant in mg. on May 9, 1929	2.2	1.9	3.7	2.7	2.3	3.0
Mean N content per plant in mg. on June 16, 1929	3.8	4.7	8.2	11.3	9.6	7.3
Yield of wheat in bushels	7.1	6.7	14.9	34.5	26.7	18.1
Yield of straw in cwt.	7.7	6.5	—	29.7	21.5	23.9

\* 1 sampling only.

† 2 samplings only.

The outstanding results of the soil analyses are the very low nitrate contents throughout the year and the relatively high ammonia contents in winter and early spring. It is quite counter to earlier experience to find consistently more ammonia than nitrate in a soil, but it appears that few determinations of ammonia have been made during cold winters. It appears that the autumn rains washed out any nitrate formed during the three months fallow on Stackyard or immediately after the ploughing in of green manures on Lansome, and that subsequently the temperature was too low for nitrification. It is to be expected that there would be less readily nitrifiable material in Stackyard than in the Lansome plots in which the green manures were ploughed in quite shortly before the wheat was sown. There is evidence in the Lansome plots but not in the Stackyard ones of slight nitrate accumulation during April and May before the period of active growth of wheat. Although the differences were small, the Stackyard tares plots gave quite consistently higher



nitrate values than the mustard plots. The ammonia contents in both fields show progressive falls from December to June (from about 8 to 1 parts of *N* per million). The ammonia contents remained high much longer in Lansome field than in Stackyard. There is evidence in Stackyard field of an increased availability of nitrogen from mustard during early summer.

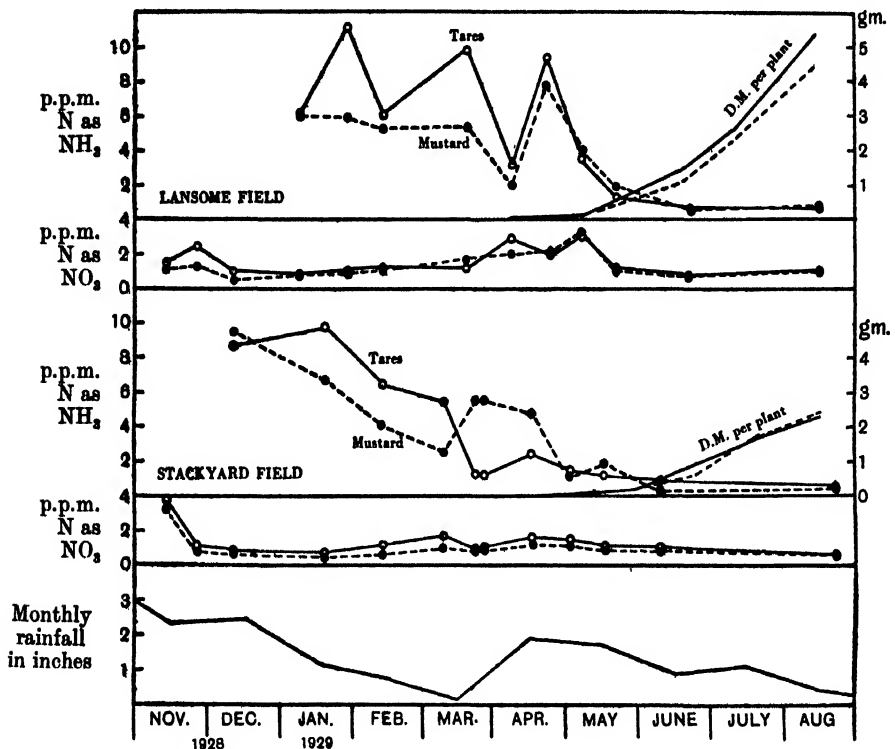


Fig. 1. Changes in ammonia and nitrate contents of soils during the growth of wheat after mustard and tares with data for the size of wheat plants and the monthly rainfalls. Woburn Stackyard field and Lansome field plots, 1928-9.

The data demonstrate a most acute nitrogen deficiency throughout the year, but the nitrate contents do not differ sufficiently to account for the wide differences in yield between the two fields. It must be remembered however that under such conditions as these, nitrate contents can give no measure of soil fertility. The nitrate measured represents the balance between production and loss by assimilation by plants and lower organisms and loss by leaching. On this light sandy soil it is clear that these losses are so high that there is no opportunity for the actual accumulation of nitrate in amounts at all comparable with those obtained in most of the published data for seasonal fluctuations in arable soils. The extra nitrogen in the Lansome field must have remained locked up

in an organic form throughout the winter and that liberated in early summer must have been taken up immediately by the wheat.

#### GREEN MANURE PLOTS, 1929.

Similar analyses were made in 1929-30 on the other half of Stackyard field starting with the first green manure crops in 1929. The field had been subjected to vigorous cultivation operations before the mustard and tares were sown (May 30). Owing to the dry summer the yield of mustard

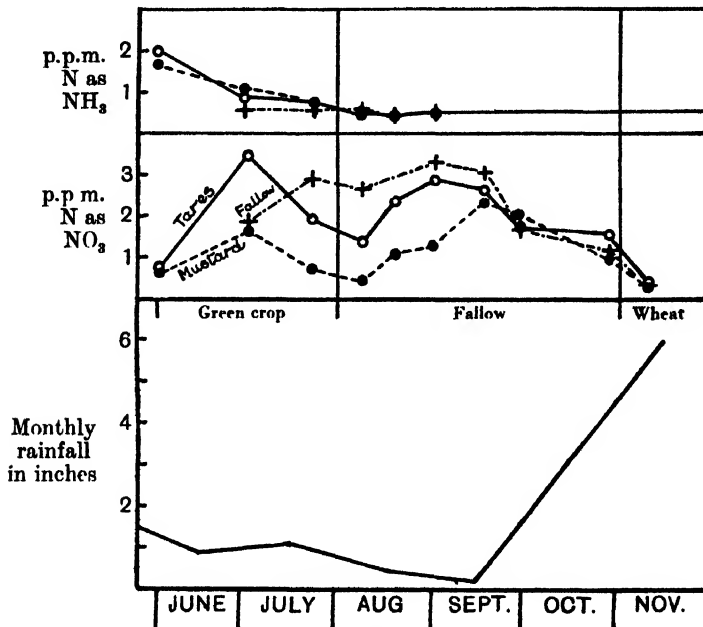


Fig. 2. Changes in ammonia and nitrate contents of soils during the growth of tares and mustard followed by fallow and wheat, with the monthly rainfalls. Woburn Stackyard field plots, 1929.

was low (2300 lb. fresh weight per acre) and that of tares was very poor (460 lb. fresh weight per acre). The crops were consumed on the land by sheep with the usual supplementary food of 3 cwt. of cotton cake per acre (July 30-August 1). The continued drought made it improbable that successful second crops could be grown and the plots were therefore fallowed with frequent harrowings. Wheat was sown early in November.

Fig. 2 gives the rainfall, ammonia, and nitrate contents for the unlimited tares and mustard plots and for an adjacent fallow plot. At the beginning of June the soil had again more ammonia than nitrate, but the ammonia contents fell off regularly and never reached amounts comparable with those of the previous year (mean value for winter 1929-30, 1.0 part N as NH<sub>3</sub> per million soil).

At the end of July the fallow strip had the highest nitrate content and the mustard plot had much less nitrate than the tares plot, as would be expected by its greater bulk and its utilisation of soil nitrogen. During the continued drought of August and September the nitrates remained almost constant in the fallow plot. In the tares plot they rose rapidly, and in the mustard plot more slowly, to give about equal values for all three plots by the end of September, when the drought broke. In all plots the nitrate content was rapidly reduced to about 1 part of nitric N per million of soil, at which value it remained throughout the year.

The second series of soil analyses adds further evidence for acute nitrogen deficiency in the wheat after green manures.

#### FIELD EXPERIMENTS 1929 AND 1930.

Direct tests for nitrogen deficiency were made in 1929 and 1930 on wheat after green manures in Stackyard field by very small scale experiments on top dressings with nitrate of soda. To secure the best comparison of the mustard and tares plots possible in absence of replication of the green manuring plots, the microplots were arranged in long narrow strips on either side of the boundary between the tares and mustard. In 1929 the produce of each plot was harvested and threshed separately and composite samples analysed. In 1930 the wheat was damaged by straying sheep in July, and the total produce was weighed without threshing. A selection from the developmental data and the final yields are given in Table II.

In both years the early applications had an immediate and striking effect in improving the colour, vigour, and tillering. Unfortunately the shoot counts in 1929 were not commenced until the maximum was passed, but the later counts show that whilst the early dressing had greatly increased the shoot numbers, the second dressing was too late to increase the number or the height of the ears, but it had greatly increased grain formation, as shown by the total yield and by the weight per ear. The essential difference between the ways in which early and late dressings act is well shown by the nitrogen percentage of the grain. Early nitrogen had no effect, showing that the nitrogen was utilised efficiently for carbohydrate synthesis and growth; late nitrogen increased the nitrogen percentage. Ear weight and nitrogen percentage in grain were closely correlated. The differences between the tares and mustard plots are relatively small in the absence of late nitrogen. Wheat after tares made less use of the late nitrogen, especially where early nitrogen had been

Table II. *Microplot experiments on Wheat after green manures.*

1928-9.

*O*=no manure.*E*=1 cwt.  $\text{NaNO}_3$  per acre on April 16, 1929.*L*=1 cwt.  $\text{NaNO}_3$  per acre on June 6, 1929.*E* + *L*=1 cwt.  $\text{NaNO}_3$  per acre on April 16 and June 6, 1929.

For each green manure plot 4 × 4 plots each 0.00135 acre.

	After tares				After mustard			
	<i>O</i>		<i>E</i>		<i>O</i>		<i>E</i>	
Shoots per metre May 14	54		64		51		71	
Shoots per metre May 29	49		63		40		61	

	After tares				After mustard			
	<i>O</i>	<i>L</i>	<i>E</i>	<i>E</i> + <i>L</i>	<i>O</i>	<i>L</i>	<i>E</i>	<i>E</i> + <i>L</i>
Shoots per metre June 24	36	34	43	42	46		46	55
Shoot height in cm. June 24	35	36	48	43	32	33	42	38
Shoot height in cm. July 18	83	82	88	85	72	77	83	87
Grain in cwt. per acre*	6.6	7.9	8.9	9.2	6.0	8.7	8.9	11.3
Straw in cwt. per acre	8.7	11.2	12.8	12.8	9.9	11.6	12.8	13.6
Ear weight in gm.	1.43	1.62	1.43	1.59	1.12	1.48	1.37	1.70
N per cent. of grain	1.34	1.56	1.32	1.56	1.27	1.47	1.22	1.52
N per cent. of straw	0.49	0.45	0.38	0.43	0.38	0.48	0.34	0.38
Percentage recovery of added N	—	27	22	21	—	41	23	34

\* Standard error: after tares 0.56 (=6.9 %); after mustard 0.75 (=8.6 %).

1929-30.

*O*=no manure.1 *E*, 2 *E*=1 cwt. or 2 cwt.  $\text{NaNO}_3$  per acre on March 8, 1930.1 *L*, 2 *L*=1 cwt. or 2 cwt.  $\text{NaNO}_3$  per acre on May 23, 1930.1 *E* + 1 *L*, 2 *E* + 2 *L*=1 cwt. or 2 cwt.  $\text{NaNO}_3$  per acre on March 8 and May 23, 1930.

For each green manure plot 3 × 8 plots each 0.00175 acre.

	Maximum shoot number per metre		
	<i>O</i>	1 <i>E</i>	2 <i>E</i>
After tares	63	76	86
After mustard	82	90	99

*Yield of total produce in cwt. per acre.*

	After tares				After mustard		
	<i>O</i>	1 <i>E</i>	2 <i>E</i>		<i>O</i>	1 <i>E</i>	2 <i>E</i>
<i>O</i>	10.2	17.2	20.6	<i>O</i>	14.6	20.5	24.9
1 <i>L</i>	18.0	19.8	—	1 <i>L</i>	21.3	26.1	—
2 <i>L</i>	20.7	—	29.6	2 <i>L</i>	22.4	—	31.5

*Summary. Yields of total produce in cwt. of wheat grain + straw per acre (average of tares + mustard plots).*

	1929			1930	
	<i>O</i>	1 <i>E</i>		<i>O</i>	1 <i>E</i>
<i>O</i>	15.6	21.7	<i>O</i>	12.4	18.9
1 <i>L</i>	19.7	23.5	1 <i>L</i>	19.7	23.0
			2 <i>L</i>	21.6	—
					30.1

given. The assimilation of the added nitrogen was very great for late applications to wheat after mustard.

In the 1930 experiment the effects on tillering are represented in Table II by the mean maximum shoot number. The general results are similar to those of 1929. Indeed for the four treatments common to the two years the wheat after mustard yields agree to about 1 cwt. per acre. The wheat after tares gave much lower yields in 1930 than in 1929.

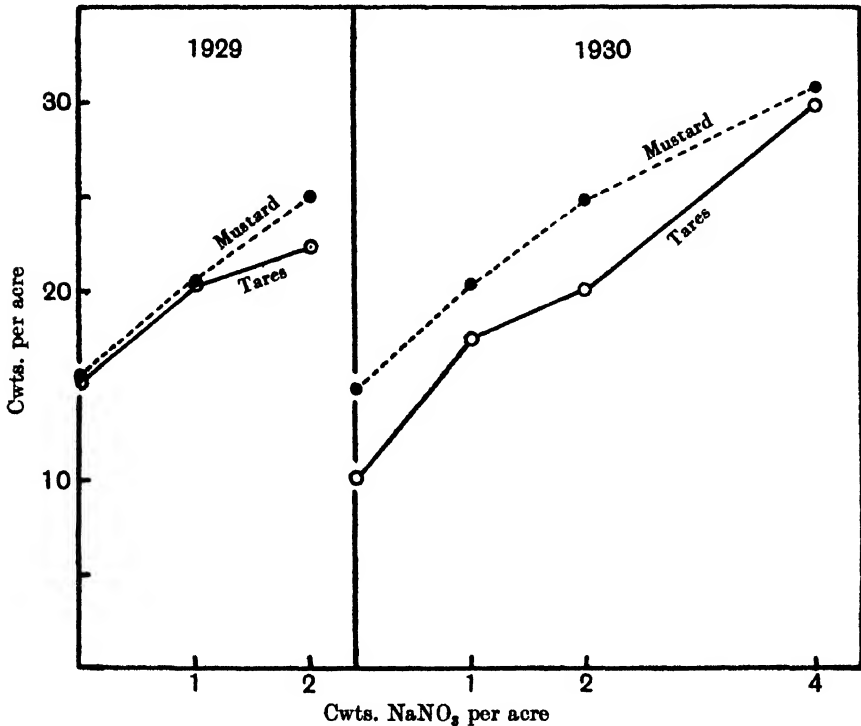


Fig. 3. Yields of grain and straw of wheat after tares and after mustard as functions of the amount of sodium nitrate given in top dressings. Woburn Stackyard field plots, 1929 and 1930.

Again the wheat after tares made little use of the single late dressing when an earlier one had been given, but it utilised the repeated double dressing as effectively as wheat after mustard.

The summary in Table II for total produce taking tares and mustard together, and that in Fig. 3 for the two crops separately, show that over a wide range the yield approximates to a linear function of the amount of nitrogen added. Although the maximum crop was still but a moderate one, there can be no doubt that, whatever the mechanism, tares and mustard fail to give a good preparation for wheat because they are

unable to provide available nitrogen in early summer at the most important period for the growth of wheat.

#### SUMMARY.

1. The Woburn field experiments on wheat after green manures are briefly reviewed. Contrary to the original expectations the wheat was less good after two summer crops of tares than after two mustard crops. This result was obtained many times and in recent years the wheat yields were extremely low after both green manures.

2. Regular soil analyses for nitrate and ammonia through 1928 and 1929 showed that the mean nitrate content was extremely low (1.2 parts of nitric nitrogen per million of soil). During the cold dry winter of 1928-9 the ammonia nitrogen was several times greater than the nitrate nitrogen.

3. Further evidence of an acute nitrogen deficiency during the critical period for the wheat plant in May and June was afforded by the large responses to top dressings of sodium nitrate both in the 1929 and the 1930 wheat crop.

4. The view is advanced that tares and mustard as summer crops fail to give a good preparation for wheat because they are unable to provide available nitrogen in early summer at the most important period for the growth of wheat.

#### ACKNOWLEDGMENTS.

The author wishes to record his indebtedness to Sir John Russell for permission to work in the Rothamsted Experimental Station; to Dr E. M. Crowther, Head of the Chemistry Department, who suggested the hypothesis that formed the basis of the work, for guidance and criticism; and to Dr H. H. Mann, Assistant Director of the Woburn Experiment Station for unfailing interest and valuable advice.

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# WINTER LEACHING AND THE MANURIAL VALUE OF GREEN MANURES AND CROP RESIDUES FOR WINTER WHEAT<sup>1</sup>.

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(With Seven Text-figures.)

ALTHOUGH systems of cropping light arable land in humid regions almost invariably involve a rotation, there is little precise information on the extent or mechanism of the effect of crops other than leys on the subsequent ones in the rotation. Precise field experiments with different rotations over periods of years are difficult to conduct, and the greatest successes in practice have been obtained by very elastic systems designed primarily to ensure a supply of sheep food at all times of the year. The most complete series of field experiments on light soils in England was commenced in 1876 by the Royal Agricultural Society at the Woburn Experimental Farm in Bedfordshire. In addition to repeating the Rothamsted continuous wheat and barley experiments it was decided to examine the residual effects of different manurial and cropping schemes throughout simple rotations. In a comparison of rich cake-fed dung with poorer corn-fed dung in a Norfolk rotation it was found, contrary to the beliefs embodied in the general farm practice of the time, that the benefit from the extra nitrogen in the cake was limited to the barley crop immediately following. Still more unexpected results were obtained in later experiments on simpler rotations. Winter wheat after summer tares (vetches—*Vicia sativa*) ploughed in was no better than wheat after summer mustard (*Sinapis alba*) ploughed in and, as the experiment continued, the yields of wheat declined very rapidly on plots with a tares-wheat rotation and rather more slowly but ultimately just as badly on the plots with a mustard-wheat rotation. Liming still further reduced the wheat after tares; mineral manures had no effect; and similar results were obtained when the whole experiment was repeated with similar summer green crops folded off by sheep which received cake in addition to the green crops. At Rothamsted, on the other hand, tares gave better results than mustard as a preparation for

<sup>1</sup> This paper is based on data presented by T. J. Mirchandani in a "Thesis approved for the Degree of Doctor of Philosophy in the University of London."

wheat. This divergence of results at the two experiment stations is typical of the conflict of experience of farmers on the relative merits of these green manures in preparation for winter cereals. An adequate explanation of the Woburn results should not only lead to the more efficient utilisation of these green-manure crops in practice but should throw some light on the more fundamental problems involved in rotations. Changes in cropping systems are being made increasingly frequently at the present time through changes in economic conditions, and many well-tried practices, such as the Norfolk rotation and its simpler modifications, must often be abandoned. Whatever the immediate results, no system can be successful if the general level of soil fertility is reduced by the unintentional inclusion of factors such as those responsible for the failure of the Woburn wheat after green manures.

Many hypotheses have been advanced from time to time to account for marked decline in fertility of the Woburn green-manuring plots, especially with tares, and some of them have already been tested in field and pot experiments with negative results. As far as we can ascertain, our own hypothesis that summer green manuring on such soil leads to an acute shortage of available nitrogen at the time of the wheat's greatest need for nitrogen has not been advanced before. The facts that relatively large amounts of nitrogen were known to be added to the soil immediately before the wheat was sown, and that the wheat started off well and for six or seven months was ahead of wheat grown in usual rotations, appear to have prevented the recognition of the nitrogen deficiency during May and June, although it has been repeatedly observed that at this time first the wheat after tares and then the wheat after mustard appear to fall behind other wheat in the same field. The results described in the preceding paper demonstrated the existence of this nitrogen deficiency by both systematic soil analyses and small scale top-dressing experiments in two seasons.

The present paper describes laboratory and pot-culture experiments on tares, mustard, and other organic materials which reproduce in part the field results and support the hypothesis that after summer green manures the lack of available nitrogen at the critical period for wheat is due to excessive losses of nitrate by leaching especially during winter. Earlier pot experiments at Woburn failed to show any inferiority of wheat after tares to wheat after mustard, but in these earlier experiments the essential factor of leaching was ignored.

In the experiments to be described one half of the pots was leached periodically from January to March and the other half was unleached.

To secure greater control of the experimental conditions the tares and mustard were not grown in the experimental pots but added in suitable amounts to uniform lots of soil.

In the stage of growth in which they are usually ploughed in or folded off in the field, tares is a soft, leafy and essentially immature plant, whereas mustard has much hard stalk; tares is rich in protein and mustard in cellulose and lignin-like products. These differences may be summarised conveniently by stating that tares has a much narrower carbon-nitrogen ratio than mustard (actually 13 : 1 and 26 : 1 respectively for the samples used in these experiments). It is well known that the oxidation of carbon compounds by micro-organisms requires a suitable supply of combined nitrogen for the production of micro-organic protoplasm. Unless the C : N ratio of plant and animal products is less than about 20 : 1 their decomposition in the soil proceeds relatively slowly unless some additional supply of combined nitrogen is available and the decomposition converts some of this available nitrogen into microbial protoplasm. Decomposition of materials with less than about 20C : 1N liberates ammonia and ultimately nitrate. On these grounds the residues of a crop of tares would be expected to decompose rapidly and liberate an appreciable fraction of its nitrogen as nitrate, whereas the residues of mustard would reduce the nitrate content of the soil for a considerable period. These differences would be still further emphasised where the crops are consumed by sheep, for the greater part of the tares is eaten, whereas much of the mustard remains as hard stalks which will be less intimately mixed with the soil mass and therefore decompose more slowly.

The main purpose of the experiment was therefore to test whether this elementary distinction between tares and mustard is sufficient to account for the differences observed in the field or whether there are more specific effects, such as a partial sterilisation from the mustard oils or a toxicity of tares, as has sometimes been suggested. The materials added to the soil were adjusted to supply the same amount of nitrogen (6 mg. per 100 gm. soil). The elementary composition of tares was imitated by a mixture of mustard and a protein and that of mustard by a mixture of tares and a source of cellulose, and both C : N ratios were also provided by mixtures of protein and cellulosic materials without green manures. Partly to utilise constituents of ordinary manures and partly to avoid exaggerated leaching effects from soluble or very readily oxidisable substances, it was decided to use blood and straw for the mixtures.

The results of two series of nitrification experiments in the laboratory and of a series of pot experiments are presented separately.

#### THE MATERIALS.

All of the plant products were air dried and finely ground in a disintegrator mill. This method of preparation may have caused changes in the compounds present in the plant products, but accurate sampling, intimate incorporation of the materials with the soil, and storage for subsequent experiments were more important than an exact reproduction of the conditions in the field on a certain date. The tares and mustard used in the major experiments were taken from the permanent green-manuring rotation plots on Stackyard field at Woburn at the end of July 1929 at the stage in which they were considered suitable for folding with sheep. In one of the laboratory experiments a sample of much younger mustard was used in addition. The farmyard manure was taken from the middle of a well-rotted heap of mixed pig and horse manure. The blood unfortunately proved to be a poor sample of low nitrogen content; the presence of some non-protein material, probably fat, may account for the relatively slow nitrification in all of the experiments. Table I gives the ultimate composition of the materials and Table II their rates of application to the soil throughout all of the

Table I. *Composition of organic materials used.*

	% N	% C	C : N
Dried blood	11.10	41.5	3.7
Young mustard	3.48	34.7	9.9
Tares	3.01	40.1	13.3
Farmyard manure	2.15	30.9	14.4
Mustard	1.51	39.9	26.4
Straw	0.32	40.9	127.8

Table II. *Mixtures supplying 6 mg. of nitrogen per 100 gm. of soil.*

C : N	Symbol		Organic matter in gm. as			Nitrogen in mg. from		
			Organic manure	Blood	Straw	Organic manure	Blood	Straw
13 : 1	<i>T</i>	Tares	0.200	—	—	6.00	—	—
13 : 1	<i>MB (T)</i>	Mustard + blood	0.170	0.031	—	2.56	3.44	—
13 : 1	<i>SB (T)</i>	Straw + blood	—	0.050	0.148	—	5.53	0.47
13 : 1	<i>F (T)</i>	Farmyard manure + blood	0.256	0.004	—	5.50	0.49	—
26 : 1	<i>M</i>	Mustard	0.400	—	—	6.00	—	—
26 : 1	<i>TS (M)</i>	Tares + straw	0.177	—	0.213	5.32	—	0.68
26 : 1	<i>SB (M)</i>	Straw + blood	—	0.044	0.344	—	4.90	1.10
26 : 1	<i>F (M)</i>	Farmyard manure + straw	0.248	—	0.206	5.33	—	0.66

experiments. These are of the same order as those supplied by the average field crops, but are much below those commonly used in ammonification or nitrification tests in the laboratory.

The soil for all of the laboratory experiments and for the major series of pot cultures was taken from a fallow plot between the green-manure plots and the continuous barley plots in Stackyard field, Woburn Experimental Station, and had been uncropped and unmanured for many years.

#### THE LABORATORY EXPERIMENTS.

It has often been demonstrated in these laboratories that the course of the microbiological activities in bottled soils is profoundly modified by variations in the degree of aeration, and that some approach to field conditions is obtained when the soil is contained in relatively large flasks and is shaken frequently to promote free gas exchange at the outside of the individual soil crumbs. Instead of taking a series of samples from a large mass of soil in a single flask a large number of separate 250 c.c. flasks containing 125 gm. of moist soil was set up so as to provide one for each analysis. In this way sampling errors were distributed at random throughout the experiment, and there was no concealed systematic change due to alterations in the degree of aeration throughout the experiment. The moisture content was adjusted to and maintained at 13.5 per cent. by frequent additions to constant weight. The flasks were plugged with cotton-wool and kept in a basement room with little daily temperature fluctuation. They were uncovered and shaken vigorously for one minute every day to ensure aeration.

Ammonia and nitrate were determined by Carsten Olsen's method (2). 100 gm. of soil were shaken for one hour with 200 c.c. of *N* KCl containing enough HCl to give a *pH* value of about 1.0. The first 25 c.c. of the filtrate were discarded and 100 c.c. of the remainder were diluted and distilled with 3 gm. of magnesia into 0.02 *N* acid and the residue again diluted and distilled with 2.5 gm. of finely powdered Devarda's alloy.

To economise space only the nitrate results are presented. With tares and other rapidly decomposing materials the ammonium nitrogen reached about 2 mg. per 100 gm. soil after 7 days and then fell to 0.1 to 0.3 mg. per 100 gm. soil after about one month. The other materials had lower initial ammonium contents and the later ones were of the same order as with tares.

The first experiment was continued for 8 months from autumn to spring and the second for 4 summer months. Figs. 1 A and 1 B show

the nitrate contents of the soils receiving single substances in the two experiments. The smoothness of the curves shows that there were no great irregularities among the flasks, and that nitrate accumulation proceeded smoothly after the first month. The rates were naturally much more rapid in the summer period and the time scale has therefore been doubled in the figure.

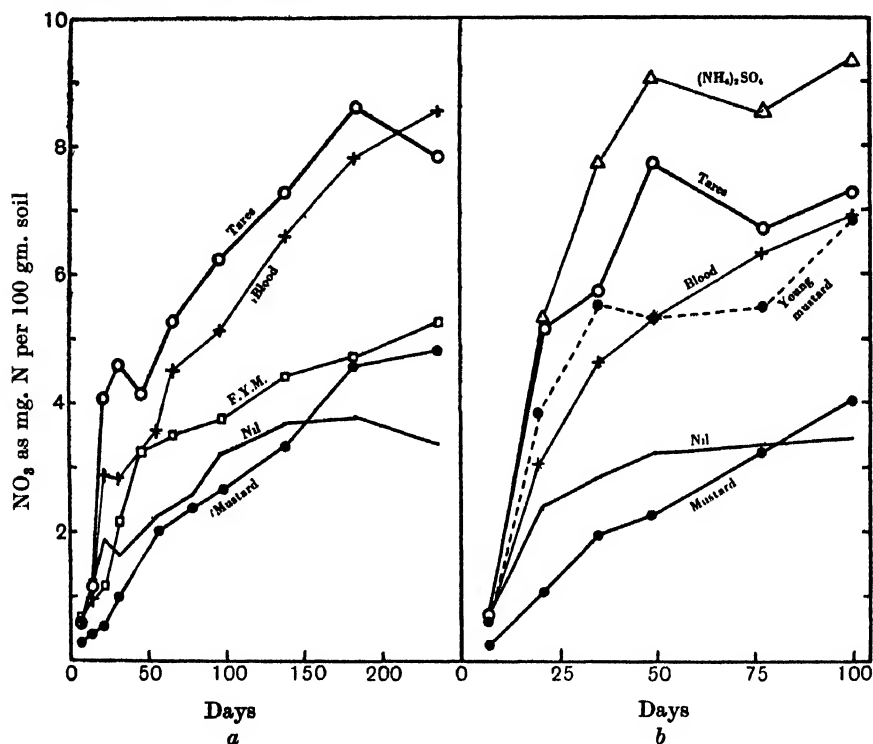


Fig. 1. Nitrate accumulation in soils receiving 6 mg. of organic nitrogen per 100 gm. soil. A, winter experiment; B, summer experiment (with expanded time scale).

#### ACCUMULATION OF NITRATE IN FLASKS.

The outstanding results are the rapidity and the extent of the nitrate accumulation from tares. The excess of nitrate over the untreated soil on the average of the last two points of the curves corresponds to 76 per cent. of the added nitrogen in the first and 62 per cent. in the second experiment. For nearly 6 months in the first experiment and throughout the whole of the second experiment tares gave more nitrate than blood, which is generally regarded as being very readily nitrifiable. In the second experiment the nitrification of tares nitrogen proceeded almost as rapidly though naturally less completely than that of ammonium sulphate. It will also be seen in Fig. 1 B that the sample of

young mustard (C : N = 10 : 1) nitrified about as rapidly as blood. Farmyard manure (Fig. 1 A) gave a steady but relatively small nitrate accumulation above that of the untreated soil after the first month. Mustard in the blooming stage, as used in the field experiments and in the pot experiments described later in this paper, immediately reduced the nitrate accumulation below that of the untreated soil and this effect lasted for some 5 months in the winter experiment and for at least 3 months in the summer one. The nitrification process was thus complete for tares before the decomposition of mustard had reached the stage at which it ceased to cause a drain on the nitrate and ammonia already present in the soil.

The results for the other mixtures used in the first experiment are set out in a condensed form in Fig. 2, which gives the means obtained by taking the 10 successive determinations together in groups of 1-3, 4-6, 7-8 and 9-10.

Each of the four sections of Fig. 2 shows the results for a pair of single substances or mixtures with C : N ratios of 13 : 1 and 26 : 1 (those for the untreated soil are added as a base line). If the C : N ratio were an adequate index of the availability of the nitrogen in organic manures the four sets of curves should be similar. Although the differences are always in the same direction, none of the other pairs shows as wide a divergence as that between the tares and mustard. With straw (Fig. 2 C) and with farmyard manure (Fig. 2 D) the differences due to C : N ratio are about one-third to one-half of those between tares and mustard. In Fig. 2 B the close similarity between the tares + straw (26C : 1N) and the mustard + blood (13C : 1N) mixtures, and the fact that the two curves run just about midway between those for tares and mustard alone, might suggest either that a specific stimulating effect of tares just balanced a specific depressing effect of mustard or, what is much more likely, that an effect due to the chemical composition or physical condition of the materials works in opposite directions at high and low C : N ratios. The essential difference between the single green manures and the mixtures is that in the former the proteins and carbohydrates occur in intimate association as relatively soft material readily open to micro-organic activity, whereas in the mixtures the proteins of the dried blood and the cellulose and lignin materials of straw occur in harder materials which are more slowly attacked and are necessarily at some distance from the particles of mustard and the tares added with them. If the decomposition of organic materials in the soil occurs as the result of associated activities amounting almost to symbiosis between a number

of groups of organisms, some liberating and others accumulating simple forms of nitrogen, it would be natural to expect this action to proceed more smoothly and rapidly where the sources of carbon and nitrogen occur in most intimate association. It was noticeable during the first month that the mixtures gave much more irregular curves than the

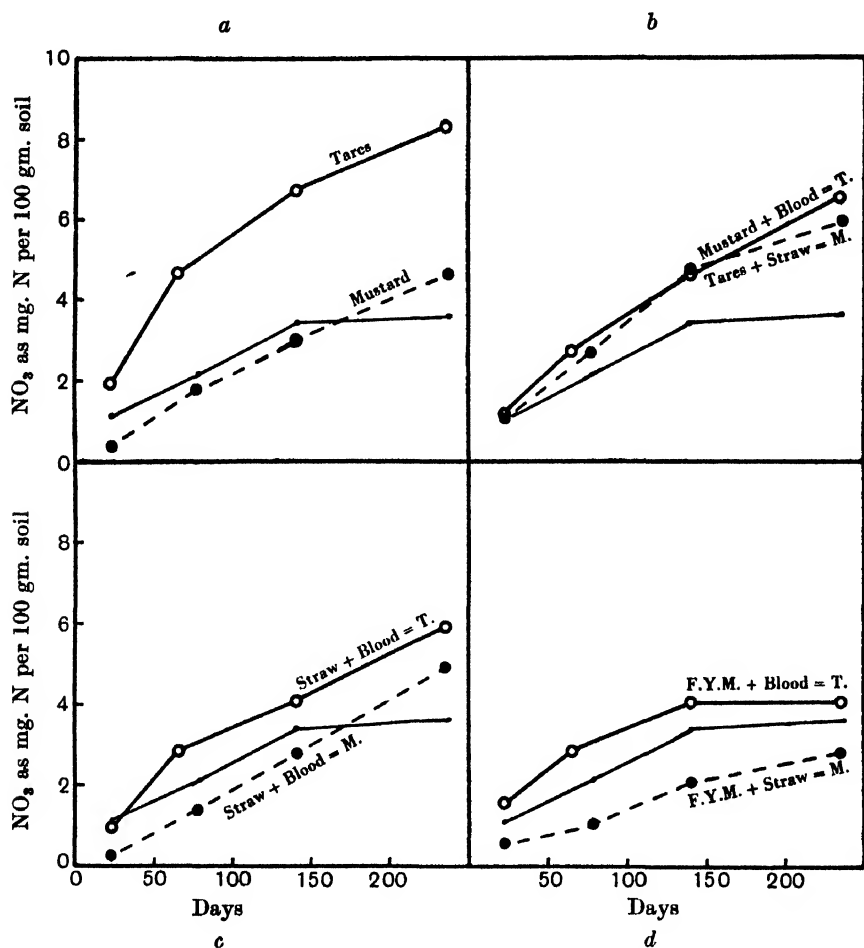


Fig. 2. Nitrate accumulation in soils receiving 6 mg. of organic nitrogen per 100 gm. soil with 13C : 1N (=tares) and 26C : 1N (=mustard) in comparison with untreated soil.

tares or mustard alone. This may be illustrated by the following figures for the variance of the three differences between successive pairs of the first four determinations of ammonia plus nitrate nitrogen:

Tares 0.18; equivalent mixtures 0.88, 0.37, 0.72.

Mustard 0.01; equivalent mixtures 0.67, 0.16, 0.72.



The greater variation between the results for the mixtures suggests that in the early stages, at any rate, there is a tendency for either the production or the consumption of inorganic nitrogen to predominate at any one time or place and that the soil mass settles down to a steady state only very slowly. This may be illustrated too by another form of expressing of the results of the second experiment, which included, in addition to the series presented in Fig. 1 B, determinations on samples of tares, mustard, and young mustard, which had been extracted with a normal solution of potassium sulphate to remove the more readily salt-soluble proteins and carbohydrates, and also series in which these extracted materials were accompanied by quite small amounts of blood. (It may be mentioned that the extracted old mustard had a much larger C : N ratio than the original material and removed almost all the nitrate; extraction of the tares and young mustard had little effect on their C : N ratios and slightly reduced the accumulation of nitrate.)

The nitrate nitrogen concentrations after 100 days are plotted in Fig. 3 against the total amount of carbon added with the standard 6 mg. of nitrogen. The results fall significantly on to a straight line such that 1 mg. of nitrate nitrogen is removed by about 30 mg. of carbon. At earlier periods there was no such regular relationship, showing that the systems were not even approaching equilibrium, as is indeed evident from the few curves plotted in Fig. 1 B. It follows from this slow approach to equilibrium that nitrogen in mixed organic manures applied in the field or in the mixtures formed by the added material and the decomposable organic matter already present as crop residues, will become available irregularly and that the course of the decomposition will be profoundly modified by comparatively small changes in environmental conditions. No very close agreement can therefore be expected between the results of experiments conducted under different conditions.

The nitrification experiments as a whole show (1) that tares liberates much of its nitrogen extremely rapidly, (2) that at first mustard locks up available nitrogen, (3) that both of these processes proceed more rapidly and completely with green manures alone than with mixtures in which part or all of the protein and cellulosic materials is derived from blood and straw respectively, and (4) that although these differences may depend in part on the actual compounds present, there is some evidence that the slowness and incompleteness of the decomposition of the mixtures depends on the less intimate association of the proteins and cellulosic bodies present in the mixtures.

To lock up equal amounts of nitrogen by mustard and by a mixture

of tares and straw requires a wider C : N ratio in the mixture than in mustard, and to secure equal liberation of available nitrogen from tares and from a mixture of mustard and blood requires a narrower C : N ratio for the mixture. The striking failure of tares in the Woburn field experiments is certainly not due to any resistance to nitrification; it may, on the other hand, be connected with its excessively rapid nitrification.

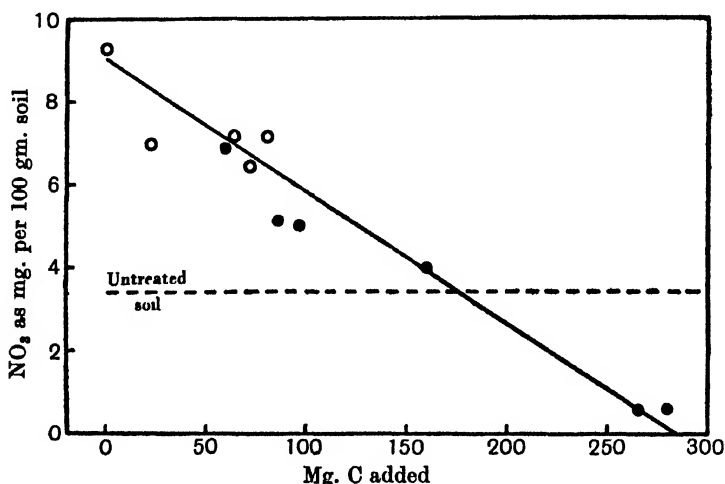


Fig. 3. Relationship between nitrate accumulation after 100 days and amount of carbon added with 6 mg. of nitrogen per 100 gm. of soil. Open circles: tares, blood and ammonium sulphate; dots: mustard, old and young.

#### POT-CULTURE EXPERIMENTS.

Pot cultures on wheat were conducted during 1929-30 at Woburn, using the same fallow-plot soil and the same forms and amounts of organic materials as in the laboratory experiments. The tares and mustard were finely ground air dried samples from the Stackyard field plots and were taken immediately before the folding by sheep in summer 1929. All additions supplied 6 mg. nitrogen per 100 gm. soil or 0.5 gm. nitrogen per pot, with amounts of carbon to give C : N ratios of 13 : 1 for tares, mustard + blood, straw + blood and of 26 : 1 for tares + straw, mustard, straw + blood.

There were three other series which received no additions: untreated fallow-plot soil was used to test the general effect due to the additions of organic matter, and untreated samples of soil from the unlimed portions of the continuous tares-wheat and mustard-wheat rotation plots in Stackyard field, Woburn were taken in October, 1929, during the cultivations for the wheat. Owing to the summer drought it had proved

impossible to grow the usual second crops of green manures, and the samples therefore contained the products from the decomposition of one crop of tares and mustard respectively during a dry period from the end of July to late October. In 1929 these soils differed from those in normal seasons in that at the time of sowing the wheat they contained no fresh tares or mustard material and no fresh sheep manure. It has been shown in the preceding paper that after folding off the tares and mustard on these plots at the end of July, 1929, the nitrate contents in the field increased rapidly on the tares plot and more slowly on the mustard plot. At the time of taking the soils for the pot experiments the three untreated soils therefore contained supplies of immediately available nitrogen with comparatively small reserves of plant residues.

The pots were made from earthenware drain pipes, 60 cm. deep and 15 cm. internal diameter, closed at the bottom by wire netting. They were filled by adding in turn, 1.5 kg. coarse gravel, 1 kg. fine gravel (passing 1 in. mesh), 8 kg. untreated soil and 9 kg. of soil containing the organic manures. All the soil had passed through a  $\frac{1}{4}$ -in. sieve and was added in 10 separate portions, each of which was lightly but uniformly pressed down. The water content was adjusted by weighings to 15.5 per cent. and maintained at this value by fortnightly waterings to constant weight with intervening waterings according to the estimated requirements. Distilled water was used throughout and the pots, though exposed in the open in the fine weather, were brought into the glass house during rain. There were 8 pots for each treatment (4 leached and 4 unleached). The 72 pots were randomised on trucks so as to eliminate place effects.

Twelve graded seeds of wheat—Little Joss W 3—were sown in each pot on November 11, 1929. From November 27 two-daily counts of the numbers of plants germinated were made until germination was complete. The results are given in Table III as percentage germinations and as the times required for three-quarters of the final number of plants to appear.

One half of each series of pots was leached with distilled water on six occasions between December 30, 1929 and April 16, 1930. The amount of water added for leaching was increased progressively as the plants gained strength, with a maximum of 3 litres of water; drainage proceeded for 2–4 days and the soil returned to normal moistness in about a week. On each occasion the leachate from each pot was measured, filtered and analysed for nitrate; tests for nitrite and ammonia showed that these were present only as minute traces (below 1 part of nitrogen

per million). One each of the untreated tares-plot and mustard-plot soils was damaged during the experiment but in the remaining 70 pots the growth proceeded quite normally throughout.

Thinning was postponed to a late date so as to reduce the risk of a loss of plants from temporary waterlogging during a hard winter. On February 28, 1930, the pots were watered and on the next day the plants were reduced to 6 per pot by pulling out the extra plants so as to remove as much root as possible. It was noticed that the plants in pots with mustard (both leached and unleached) had much longer and more abundant root systems than those with any of the other treatments. The plants from each treatment (separating leached and unleached) were cleaned, mixed, dried, weighed, and analysed for total nitrogen. By making the assumption that the plants removed were similar to those left in the pots it was possible to estimate the dry matter and nitrogen contents of the young wheat plants at the time of thinning (March 1).

Observations on the development of the plants taken periodically included frequent counts of the number of plants, and shoots per pot, and the height of each shoot to the base of the top unfolded leaf. In the statement of results the shoot numbers before thinning are reduced to a constant basis of 6 plants per pot. The shoot heights are recorded by giving the time at which half of the final value was reached. During the later stages of growth there were striking differences in the rates of ear emergence. Two-daily counts of the fully emerged ears were made, and the results are summarised by recording the times at which one-half of the final number of ears had emerged.

The wheat was harvested on August 18, 1930, and the produce of each pot was treated separately throughout in order that valid estimates of standard error could be made not only for yield of grain and straw (including chaff and husk) but for each of the quantities: number of ears, number of grain, nitrogen percentage and total nitrogen content of grain and of straw.

#### THE REMOVAL OF NITRATE BY LEACHING.

Table III gives the mean nitrate nitrogen content of each of the leachings. The standard error of the total nitrate was calculated for the fallow-plot soils only (28 pots) as one pot was lost from each of the tares-plot and mustard-plot soils.

The average total nitrate content of the leachates from the three sets with 13C : N was 85 mg. and that from the three with 26C : N was 60. The difference (25) may be regarded as significant, as the standard

error for these means of 12 pots is 5.8. Considering the individual results, it will be seen that both mustard and tares + straw significantly depressed the nitrate loss below that from the untreated soil. The other differences for the fallow-plot soil cannot be regarded as significant in view of the high standard error.

Table III. *Amount of nitrate nitrogen in mg. in pot in total leachate.*

Leaching		Soils from green-manure plots			Soil from fallow plot with added organic matter					
		No addition			+ 13C : 1N			+ 26C : 1N		
Date	Amount in litres	Tares plot	Mustard plot	No addition	Tares	Mustard + blood	Straw + blood	Mustard	Tares + straw	Straw + blood
31. xii. 29	1.0	14	6	2	3	2	3	2	3	2
17. i. 30	1.5	62	57	24	24	24	21	15	15	25
4. ii. 30	2.5	32	19	40	40	38	32	22	21	35
22. ii. 30	2.5	7	8	6	22	16	11	7	7	12
19. iii. 30	2.5	2	2	3	7	4	2	2	3	4
16. iv. 30	3.0	2	4	3	3	2	1	2	1	2
Total		119	96	78	99	86	70	50	50	80

Standard error of the totals 8.2 (or 11.3 per cent. of the general mean).

Both the tares-plot and the mustard-plot soils gave very high nitrate contents, especially in the earlier leachings. The second leaching contained more than twice as much as that from any other soil and one-half of the total from these soils. As these were both leachings with small amounts of water, it is clear that at the time of sowing wheat there was much readily available nitrogen from both the tares and mustard folded off three months before.

The low nitrate contents of the last two leachings is ample evidence that by this time the combined action of plant and drainage had reduced the nitrate concentration to a very low level, and that any available nitrogen formed in March and April was immediately assimilated by the rapidly growing plants.

It should be pointed out that in a normal English autumn and winter extensive drainage commences much earlier than the leaching in these experiments and the losses of nitrate in the field are probably much more serious than in our pots.

#### THE DEVELOPMENT OF THE CROP.

##### *Germination.*

Within the first fortnight of the experiment differences between the tares and mustard treatments were apparent in the rates of germination which are recorded in a condensed form in Table IV. In all cases the

series with 13C : N ratios gave less complete and less rapid germination than those with 26C : N. Very soon afterwards, however, the plants with manures of the lower C : N ratio caught up with the other series and grew much more rapidly. Although it has not proved possible as yet to examine the effect on germination more closely, it has undoubtedly practical and scientific interest. It has been suggested to us by experienced observers that one of the reasons for the poorness of wheat after tares may be found in its poor germination owing to the dryness of the soil after tares. This explanation is obviously not applicable to our experiments, for special measures were taken to keep the surface of the soil uniformly moist during the period of germination. It is indeed possible that the poor germination observed by farmers is due to some such effect as that in our pot experiments. It is well known that the accumulation of carbon dioxide or ammonia may inhibit the germination of seeds and Fred (3) has shown that certain organic manures may have an adverse effect through causing a large growth of fungi. Although he did not find this effect with wheat, he did not experiment with tares which decompose with extreme rapidity and allow the accumulation of ammonia, carbon dioxide, and fungi.

Table IV. (a) *Final germination as percentage of seeds sown.*

	Uncropped soil			
	Green manures added	Green manure mixtures added	Straw + blood added	Field-plot soils No addition
13C : 1N, tares equivalents	86.5	84.4	84.4	81.6
26C : 1N, mustard equivalents	88.5	86.1	92.5	87.5
Difference 13C-26C	-2.0	-1.7	-8.1	-6.4

(b) *Time in days required for germination of 75 per cent. of final number of seedlings.*

13C : 1N, tares equivalents	18.6	17.8	17.3	16.8
26C : 1N, mustard equivalents	17.2	16.5	15.9	16.2
Difference 13C-26C	1.4	1.3	1.4	0.6

### *Tillering.*

Shortly after germination the plants in the mustard series, whether leached or unleached, fell far behind the others. The plants were small single shoots with narrow leaves and a bluish grey colour. Growth and tillering proceeded actively in the unleached soils in the tares equivalent series and in the untreated soils and much more slowly in the leached

series. The whole course of development is illustrated in Fig. 4 for the leached and unleached tares and mustard treatments. With unleached tares tillering proceeded steadily in January and February and rapidly in March to a maximum of 6 shoots per plant, whereas with leached tares there was a much slower rise to a maximum of 3 per plant. With mustard tillering was very slow; until March there were no tillers in either series, and the maximum shoot numbers (2.7 per plant) were not reached until several weeks after the tares had reached its maximum.

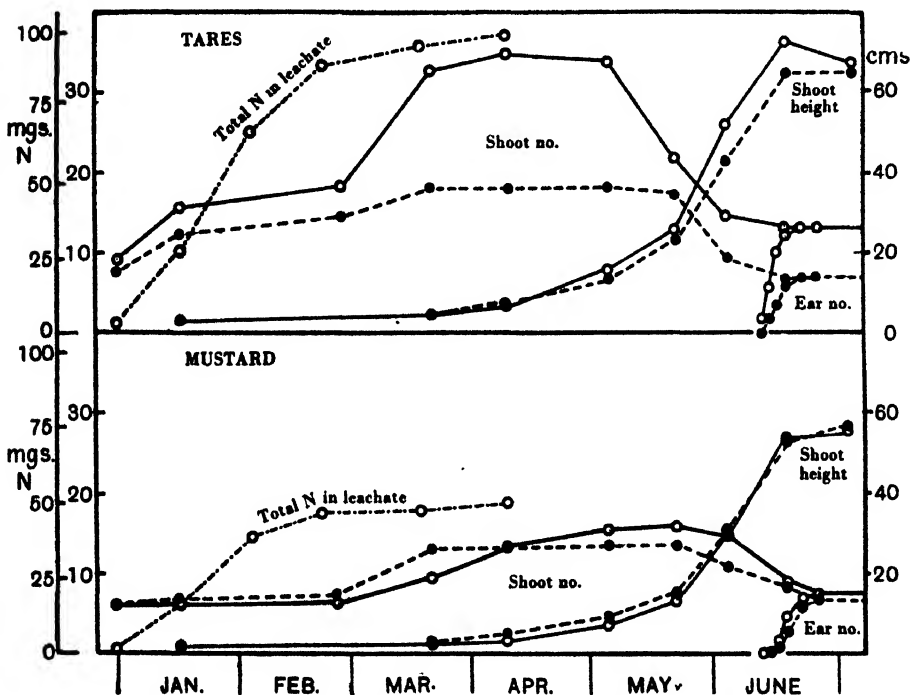


Fig. 4. Developmental data for wheat in pots manured with tares and mustard respectively. Full lines for unleached pots and broken lines for leached.

In the earlier stages the unleached series was actually below the leached series in tiller numbers. The shortage of available nitrogen in the mustard series is in harmony with the nitrate content of the leachings and is reflected in the low final ear numbers.

Mean values of the data for all of the treatments are given for several of the more important stages of development in Table V and for the final yields and nitrogen contents in Table VI. To facilitate comparisons between the different treatments at successive stages of growth, the general forwardness or backwardness of the plants is represented in Fig. 5 by plotting the deviations of the treatment mean from the general

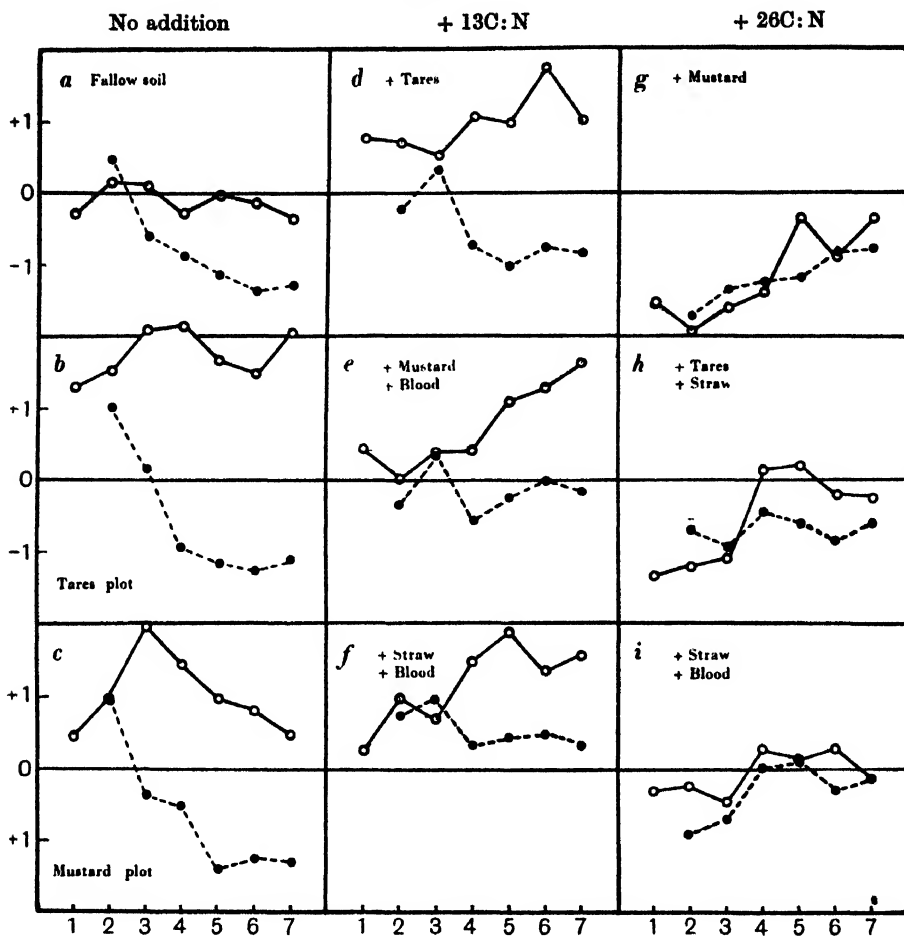


Fig. 5. Comparison of the rates of development and final yields of wheat in unleached pots (full lines) and leached pots (dotted lines) for (1) three series of soil without added organic matter, (2) three with added organic matter equivalent to tares, and (3) three with added organic matter equivalent to mustard. Deviations from general mean are given in terms of the standard deviation of the means for 18 treatments. Abscissae 2-6 represent successive stages of development as shown below.

	Date	Mean value <i>M</i>	Standard deviation s.d.	s.d. as % of <i>M</i>
1. Nitrogen in mg. in leachate and leached plants at thinning	1. iii. 30	97	28	29
2. Shoot numbers, early period, during leaching	1. i. 30-27. ii. 30	12.1	3.1	25
3. Nitrogen in mg. in young plants at thinning	1. iii. 30	24.6	12.1	44
4. Shoot numbers, middle period	22. iii. 30-6. v. 30	24.5	8.6	35
5. Shoot numbers, late period	23. v. 30-20. vi. 30	13.9	2.6	19
6. Grain and straw in gm.	18. viii. 30	25.8	7.3	28
7. Nitrogen in mg. in grain + straw	18. viii. 30	196	53	27



mean, as multiples of the standard deviation of the 18 treatment means. This makes it possible to compare dissimilar quantities such as tiller numbers and nitrogen contents on a uniform basis. The general means and standard deviations used in preparing Fig. 5 are given below the figure.

Some of the individual stages will be discussed first. In dry weights and nitrogen contents of the young plants at the time of thinning (Table V, rows 2 and 3; Fig. 5, abscissae 3), it will be seen that leaching had an appreciable effect only on the two untreated soils from the green manure plots. It has already been shown that these contained much larger amounts of immediately available nitrogen than the fallow-plot soils with added organic matter. Some of this immediately available nitrogen was thus removed before the young seedlings could absorb it. In all of the series in fallow-plot soil the leached plants had slightly more dry matter, but the same total nitrogen contents as the unleached ones. Although leaching had thus little or no effect on the absorption of nitrogen before March 1, there were striking differences between the effects of the added organic materials. Each of those with 13C:1N gave a higher nitrogen content than the untreated soil and each of those with 26C:1N gave a lower nitrogen content. Mustard treatment reduced the nitrogen content of the young plants to one-fifth of that of the unmanured plants when unleached and to one-half when leached. In all cases the amount of nitrogen in the young plants was less than half and in some as little as one-sixth of that removed in the leachate. This is one of the most important results of the experiment, for it amply demonstrates the inability of young wheat plants to utilise the large amounts of nitrogen often made available during the autumn and early winter. Under field conditions the losses are likely to be greater than in these experiments, for the cultivation for the wheat seed-bed favours active nitrification and leaching, whilst in these experiments the green manures were added at the time of sowing and leaching was not commenced until 7 weeks later.

By adding together the total nitrogen contents of the leached plants (including the ones removed at thinning) on March 1 and the total nitrogen removed in the leachates up to this date it is possible to obtain an estimate of the total available nitrate produced by this date. The results are given in Table V, row 4 and in Fig. 5, abscissae 1 (they differ but slightly from those given by adding rows 1 and 3 in Table V, for the amount of nitrate subsequently leached was small and of the same order as the nitrogen in the few plants removed at thinning). Mustard

Table V.

	Untreated-field soils				Uncropped-plot soil with added organic matter						s.e. for (d) to (i) (figures in brackets give s.e. as % of general mean)
	Un- cropped				13C : 1N			26C : 1N			
	Tares plot (a)	Mustard plot (b)	cropped plot (c)	Un- cropped plot (c)	Tares (d)	Mustard + blood (e)	Straw + blood (f)	Tares + straw (g)	Mustard (h)	Straw + blood (i)	
1. Nitrate nitrogen in total leachate, mg.	L	118	96	78	99	86	70	50	50	80	8.2
2. Weight of plants left at thinning, gm.	U	1.04	0.91	0.46	1.10	0.85	0.85	0.37	0.33	0.48	(11.3)
3. Nitrogen in plants left at thinning, mg.	L	0.86	0.76	0.68	1.16	1.31	1.34	0.40	0.31	0.80	—
4. Nitrogen available by 1. iii. 30 (nitrogen in total leached plants + leachate by 1. iii. 30)	L	50	45	26	30	29	33	12	5	19	—
5. Shoot number, early period (1. i. 17. i, 27. ii)	L	27	20	17	29	28	37	13	8	16	—
6. Shoot number, middle period (22. iii, 16. iv, 6. v)	L	161	125	102	136	131	130	70	61	102	—
7. Shoot number, late period (23. v, 5. vi, 20. vi)	U	16.8	15.0	12.6	14.3	12.1	15.1	8.4	6.1	11.3	—
8. Time for half shoot height (for days ahead of general mean)	L	15.2	15.0	13.5	11.4	11.0	14.3	10.0	6.8	9.3	—
9. Time for half ear emergence (for days ahead of general mean)	U	42.7	36.9	31.9	33.9	27.8	37.2	25.4	12.7	26.7	2.15
10. Number of ears	L	16.3	18.8	16.8	18.2	19.9	14.3	20.8	13.1	24.7	(9.2)
11. Number of grain per ear	U	15.0	16.4	13.8	16.4	16.8	18.8	14.4	13.0	14.3	—
	L	10.9	10.2	10.9	11.2	13.3	15.0	12.2	10.7	14.2	—
	U	2.2	2.3	2.5	2.5	1.7	1.7	1.9	2.5	0.1	—
	L	1.9	2.9	3.7	1.2	1.5	3.6	1.2	3.1	0.8	—
	U	1.7	1.0	0.4	1.8	1.2	1.5	1.4	1.5	0.8	—
	L	1.2	1.8	3.0	0.5	0.8	1.6	1.4	3.0	0.4	—
	U	12.2	11.8	8.5	13.0	12.5	11.8	8.0	7.5	9.3	—
	L	6.0	6.0	6.0	6.5	8.8	11.0	6.8	6.5	7.3	—
	U	21.5	21.2	24.2	19.1	22.4	22.6	22.5	22.9	24.2	—
	L	23.3	23.0	23.6	24.7	21.1	21.0	23.2	24.8	26.2	—

U = Unleached series; L = Leached series. All values as means per pot of 6 plants.

Table VI.

	Untreated-field soils				Uncropped-plot soil with added organic matter					s.e. for (d) to (i) (figures in brackets give s.e. of as % of general mean)
	Tares plot		Un- cropped plot (c)	13C : 1N		28C : 1N				
	(a)	(b)		Tares (d)	Mustard + blood (e)	Straw + blood (f)	Tares + straw (g)	Mustard (h)	Straw + blood (i)	
1. Grain, gm.	U 10.6	11.2	8.8	10.0	11.4	11.3	8.0	7.7	9.8	0.35
	L 6.3	6.6	5.8	6.8	8.9	9.1	6.8	7.5	8.2	(4.0)
	U-L 4.3	4.6	3.0	3.2	2.5	2.2	1.2	0.2	1.6	—
2. Straw, gm.	U 25.9	20.2	16.9	28.5	23.9	24.4	16.2	13.5	18.0	1.10
	L 10.4	10.1	10.0	13.6	16.9	20.2	13.1	12.3	15.5	(6.1)
	U-L 15.5	10.1	6.9	14.9	7.0	4.2	3.1	1.2	2.5	—
3. Grain + straw, gm.	U 36.5	31.4	25.7	38.5	35.3	35.7	24.2	21.1	27.9	0.02
	L 16.7	16.7	15.8	20.4	25.8	29.3	19.7	19.9	23.7	(3.4)
	U-L 19.8	14.7	9.9	18.1	9.5	6.4	4.5	1.2	4.2	—
4. Nitrogen in grain, mg.	U 190	151	126	150	175	181	128	136	150	7.0
	L 105	104	97	111	144	148	118	121	136	(5.0)
	U-L 85	47	29	39	31	33	10	15	14	—
5. Nitrogen in straw, mg.	U 115	68	51	99	107	96	56	46	54	8.1
	L 34	24	31	43	44	63	47	35	51	(13.1)
	U-L 81	44	20	56	63	33	9	11	3	—
6. Nitrogen in grain + straw, mg.	U 305	219	177	249	282	277	184	182	204	18.0
	L 139	128	128	154	188	211	165	156	187	(9.0)
	U-L 166	91	49	95	94	66	19	26	17	—
7. Nitrogen per cent. of grain	U 1.82	1.36	1.35	1.51	1.53	1.61	1.60	1.65	1.53	0.015
	L 1.65	1.57	1.68	1.64	1.62	1.62	1.82	1.61	1.67	(0.90)
	U-L 0.46	0.32	0.27	0.35	0.44	0.40	0.35	0.34	0.30	0.038
8. Nitrogen per cent. of straw	U 0.31	0.24	0.31	0.32	0.26	0.31	0.34	0.29	0.33	(11.3)
	L 0.29	0.36	0.34	0.26	0.32	0.32	0.33	0.36	0.35	—
	U-L 0.38	0.40	0.37	0.33	0.34	0.31	0.34	0.38	0.35	—
9. Ratio of grain weight to grain + straw weight	U 0.62	0.69	0.71	0.60	0.62	0.65	0.70	0.75	0.74	—
	L 0.76	0.81	0.76	0.72	0.77	0.70	0.72	0.78	0.73	—
10. Ratio of nitrogen in grain to nitrogen in grain + straw	U 0.76	0.81	0.76	0.72	0.77	0.70	0.72	0.78	0.73	—

All values as means per pot of 6 plants.

U = Unleached series; L = Leached series.

and (tares + straw) gave less available nitrogen, and each of the 13C : N series gave more available nitrogen than the corresponding untreated soil. The tares-plot soil gave considerably more early available nitrogen than the mustard-plot soil.

The forwardness and the healthy green colour of the plants in the green manure-plot soils and in the fallow-plot soil with tares equivalents were very striking at this stage for both leached and unleached soils. This is illustrated by the early shoot numbers (Table V, row 5, and Fig. 5, abscissae 2) which are high for these treatments and resemble the nitrogen contents of the young plants in showing no consistent leaching effect.

From the time of thinning the effects of leaching become much more striking in all of the series except those receiving organic manures with 26C : 1N. The rapid tillering illustrated in Fig. 4 for the unleached tares series also occurred in the unleached tares-plot and mustard-plot soils and in the other unleached 13C : N series, whereas the corresponding leached pots tillered much more slowly even though for the 13C : N series there was no difference in nitrogen content at the time of thinning. The effect of previous leaching is well shown in Figs. 5 D, 5 E, 5 F, by the increasing divergence of the curves for leached and unleached 13C : N series. The greatest difference between leached and unleached series was for the tares-plot soil. In the 26C : 1N series the differences between leached and unleached series were small and inconsistent. Both mustard series started off much below the others but gained steadily, until in final yields they were ahead of the leached tares and the three leached untreated series. The other series with 26C : 1N caught up with the general mean by the time of maximum tillering (abscissae 4). The actual numbers of shoots in Table V show that in the unleached tares-plot and mustard-plot soils and the unleached soil with added tares the late shoots were about equal to those of the early period, whereas in the leached soils the late shoots were less than those in the early period. In the unleached series enough nitrogen was available to carry to maturity most of the tillers originally formed, but in the leached series there was a nitrogen shortage in the later stages of growth. With mustard and with all of the mixtures (in both leached and unleached series) the late shoot numbers were greater than the early shoot numbers, indicating that the nitrogen shortage was less acute in the late than in the early stages of growth.

The use of shoot numbers as approximate measurements of the amount of nitrogen available to the plants is based on plant physio-

logical evidence that the nitrogen supply limits the production of meristematic tissue when other environmental conditions are suitable for growth. Up to the point of maximum shoot numbers nitrogen is absorbed rapidly and stored temporarily in highly nitrogenous young shoots, but when the supply of nitrogen to the roots falls below that required to meet the requirements for the growth of the larger and older shoots, nitrogen is translocated to them from the smaller shoots which die off. The intimate connection between shoot numbers and the nitrogen contents of the plants is illustrated for the present experiments in Fig. 6. At the time of thinning the shoot numbers and the nitrogen contents are very closely correlated, and again at harvest there is a fairly close correlation between ear numbers and the total nitrogen in grain and straw, though there is some evidence of an upper limit of 2 ears per plant. The correlation for the mature plant is also expressed by the fact that the nitrogen percentages of the grain and straw and the ratios of grain to straw vary much less among themselves than do the actual yields. We have no determinations of nitrogen contents between thinning and harvest, but the correlation of maximum shoot numbers and final nitrogen contents is in agreement with the view that the plant absorbs substantially the whole of its nitrogen by the time of maximum tillering.

Fig. 6 also illustrates the important fact already mentioned that, although no appreciable amount of nitrate was removed by leaching after thinning, the effects of leaching were entirely masked at the time of thinning and became prominent only several weeks later when the plants had taken up much more nitrogen. The effects of leaching on the subsequent availability of nitrogen appear to be more important than the actual removal of nitrate, though it will be shown later that these two effects are closely correlated under the conditions of these experiments.

It was observed that where there had been an early supply of available nitrogen (tares-plot soil, mustard-plot soil, and fallow-plot soil with tares and in both leached and unleached series in each case) the plants were ahead of the others in the length of the shoots and in time of ear formation even though the leached pots gave much fewer shoots and ears. When the data in rows 8 and 9 of Table V for the earliness or lateness of shoot and ear formation were plotted on curves similar to those in Fig. 5, they fell quite out of line with the other developmental data. The relative importance of the early nitrogen and the total nitrogen in determining the rate of development of the shoot is shown in Table VII, which gives the correlation coefficients for the time of

emergence of half the ears and the nitrogen contents at the times of thinning and at harvest respectively. (The times for half-shoot height were closely correlated with those for half-ear emergence.)

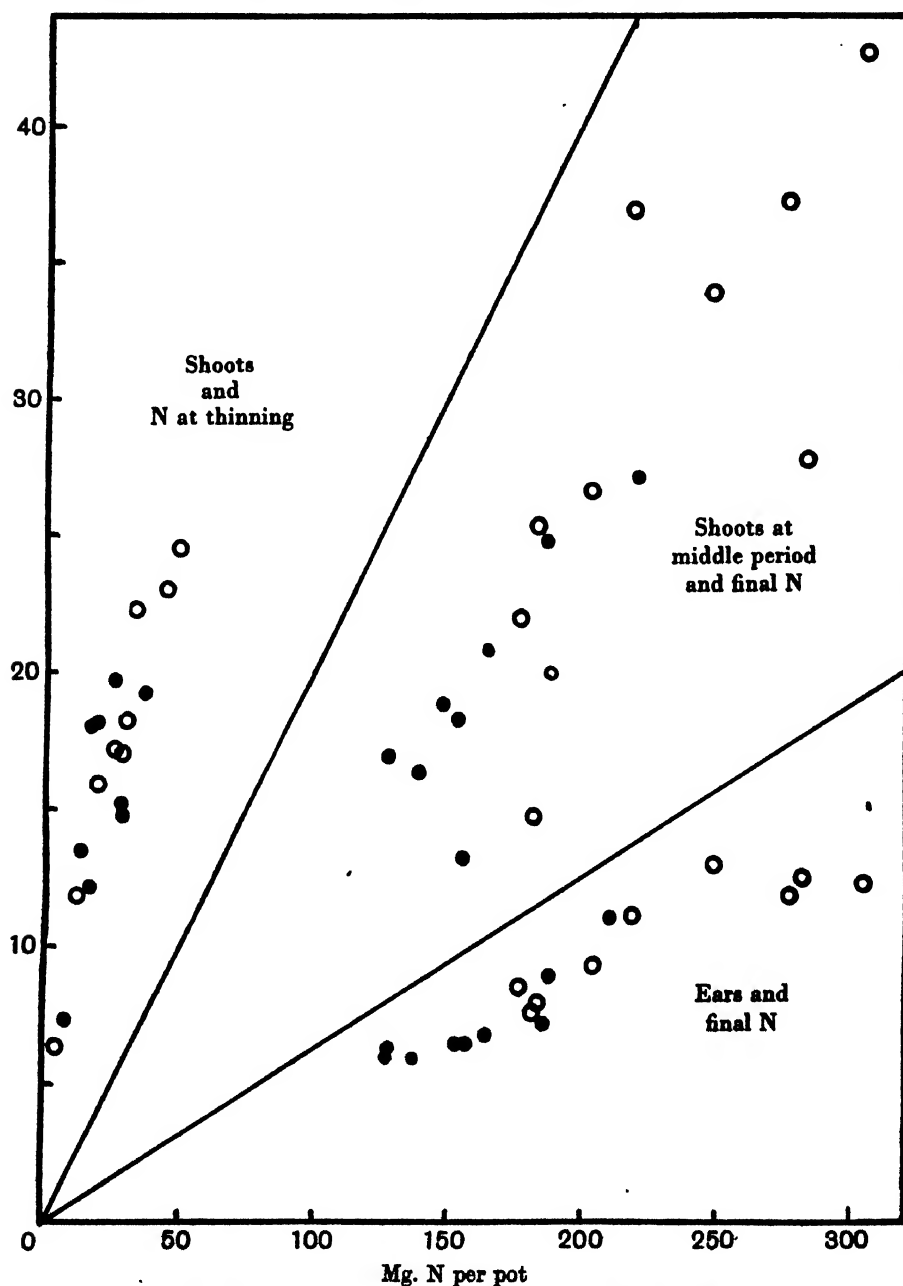


Fig. 6. Relationships between shoot numbers and nitrogen contents at different stages in the growth of wheat. Open circles for unleached pots; dots for leached pots.

Table VII. *Relationship of times of ear emergence to nitrogen contents of the plants.**a* = nitrogen content of plants per pot at thinning.*b* = nitrogen content of plants per pot at harvest, grain + straw.*c* = time of emergence of half of final number of ears ( - equals ahead of general mean).

		Correlation coefficient			Percentage probability of similar effect by chance		
		All	Un-leached	Leached	All	Un-leached	Leached
$r_{ab}$	Early N and final N	+0.58	+0.71	+0.21	1	<5	High
$r_{ac}$	Ear emergence and early N	-0.78	-0.98	-0.73	<1	<1	<5
$r_{bc}$	Ear emergence and final N	-0.72	-0.78	-0.74	<1	<5	<5
$r_{ac \cdot b}$	Ear emergence and early N eliminating final N	-0.64	-0.96	-0.88	<1	<1	<1
$r_{bc \cdot a}$	Ear emergence and final N eliminating early N	-0.53	-0.59	-0.89	<5	High	<1

\* The difference between these pairs of correlation coefficients is significant, i.e. ear emergence in the unleached series is connected more closely with the early nitrogen than with the final nitrogen.

The nitrogen contents at thinning and at harvest are correlated positively in the unleached series but not in the leached series. Early ear emergence is associated with high nitrogen contents both at thinning and at harvest, but the partial correlation coefficients show that in the unleached series time of ear emergence and early nitrogen are very highly correlated, and further that the time of ear emergence is more closely connected with the early than with the final nitrogen. In the unleached series supplies of nitrogen during active growth varied widely from treatment to treatment, but they were always adequate to allow the plant to develop at a rate determined by the initial supply. In the leached series the nitrogen subsequently became available at more widely different rates according to the treatments; the early and the final nitrogen contents were almost independent but they proved to be equally connected with the time of emergence of the ears. Low availability of nitrogen during the early stages was compensated for by greater supplies later from the soils which had lost least by leaching, whereas high initial availability gave large early plants whose subsequent development was retarded by nitrogen shortage later in growth.

## STATISTICAL ANALYSIS OF FINAL YIELDS.

The experiment was designed primarily to test whether the essential differences between tares and mustard were to be ascribed to their elementary composition or to specific differences. These questions may be tested by averaging the results for the two C : N ratios and also by taking together the following pairs of treatments: (tares and tares + straw), (mustard + blood and mustard), (straw + blood (= *T*) and straw + blood (= *M*)). On the first hypothesis the effect of leaching should vary significantly with the C : N ratio, and there should be no significant interactions between each of the three main sources of organic matter and the effects of leaching, amount of carbon, and the interaction of leaching and amount of carbon and also no effect on the mean yield. Such tests are conveniently made by R. A. Fisher's Analysis of Variance which uses the degree of agreement between the replicates to estimate the probability that the observed differences may be ascribed to chance variations and experimental errors. In Table VIII the mean squares

Table VIII. *Analysis of variance of final yields.*

	Degrees of freedom	Sums of squares (for mg. N in plant)	$e^{2z-2z'}$ where $z'$ corresponds to $P=0.01$ . Significant results italicised					
			mg. N in			gm. dry matter in		
			Plant	Grain	Straw	Plant	Grain	Straw
1. Leaching	1	32,761	3.4	4.6	5.2	24.6	11.9	7.9
2. Amount of C	1	31,930	3.3	3.3	4.3	30.1	8.2	13.4
3. Leaching and amount of C	1	12,871	1.3	1.0	2.8	8.5	2.0	3.8
4. Form of C	2	9,057	0.7	3.0	0.2	3.5	5.1	1.4
5. Form of C and leaching	2	721	0.1	0.0	0.3	2.3	0.4	1.5
6. Form of C and amount of C	2	5,127	0.4	0.5	0.3	0.8	1.2	0.2
7. Form of C, amount of C and leaching	2	445	0.0	0.0	0.2	2.2	0.5	0.8
8. Error	36	46,916						
9. Total	47	139,828						
	s.e. per pot	36.1						

due to treatments and to error are given for one of the variates tested (viz. nitrogen content of the grain and straw in mg. per pot), but instead of customary practice of recording the " $z$ " values those for  $e^{2z-2s'}$  are used. This quantity expresses the ratio of the observed mean square due to treatment to that required to give a 1 per cent. probability that the effect arose by chance. All values of  $e^{2z-2s'}$  which exceed 1.0 may therefore be taken as highly significant, and those which exceed 0.7 ( $P = 0.05$ )



may also be regarded as significant. This method of expression has the advantage that it provides a ready means for comparing several variates from the same experiment in a simple table. The results of the analysis of the variance for the final yields of grain and straw and for grain + straw and for their nitrogen contents are given in Table VIII in this form. The analysis is restricted for simplicity to the 48 pots to which organic materials were added, and the standard errors for the means given in Table VI are subject to similar restrictions.

The results for total nitrogen in grain + straw are considered first. These show that both leaching and the C : N ratio have had highly significant effects and that the interaction between them is also highly significant. Inspection of the individual means in Table VI shows that leaching markedly depresses the nitrogen recovery from tares equivalents but not from mustard equivalents. This fully confirms the original hypothesis on which the work was based. Specific effects due to the various forms of carbon do not attain the level required for significance ( $P = 0.01$ ), but there is an indication that the general level of nitrogen recovery depends on the form of carbon, and in the detailed results mixtures of straw and blood are generally better than tares or tares + straw. The analyses of the other yield data confirm those for nitrogen recovery. Owing to the better agreement between replicates the yields of dry matter show still more striking effects for leaching, amount of carbon, and their interaction. For every variate tested these effects are highly significant and always depend on the superiority of unleached tares equivalents over leached tares equivalents and over mustard equivalents, whether leached or unleached. There are in addition to this main effect a few significant specific effects which can be summarised as follows:

1. In the nitrogen content of grain, tares and tares + straw give lower values than other materials of the same C : N ratio whether leached or unleached.
2. In dry weight of grain, tares alone gives less than other materials of 13C : N whether leached or unleached.
3. In dry weight of straw, mustard alone and mustard + blood are less influenced by leaching than the other materials.
4. In dry weight of grain + straw, leaching has more effect on tares alone and less effect on mustard alone than on equivalent mixtures. The third and fourth results are in harmony with the original hypothesis with a modification brought out by the laboratory experiments, viz. that tares gives more and mustard less rapid and complete nitrifi-

cation than equivalent mixtures. The inefficiency of tares for grain production brought out in the first and second additional significant interactions is not expected from the original hypothesis, though it resembles one of the outstanding features of the Woburn field experiments. It also happens that untreated soil from the tares plot gives less grain than that from the mustard plot, but this difference is small and uncertain. Comparison with the untreated fallow-plot soil shows that in the unleached series the addition of tares increased the straw by two-thirds but the grain by only one-seventh. In each of the three sets, untreated soils, soils with addition of 13C : N, and soils with addition of 26C : N, the ratios of grain to grain + straw and of nitrogen to grain in nitrogen in grain + straw are lower for soils receiving tares in some form than for the other soils. In the unleached series with 13C : N the tares produced rather more ears than the other materials but 10 per cent. less grains per ear.

It is not possible from these experiments to explain the abnormality of tares when no leaching occurs. There are indications that it arises from an excessively rapid availability of nitrogen leading to too rapid development of the plants and an insufficiency of available nitrogen in the later stages of growth. It is, however, quite clear that the major difference between tares and mustard lies in the greater losses from tares through winter leaching and also that any additional effects are due to the abnormality of the tares rather than to an abnormality of the mustard as has sometimes been supposed in discussions on the Woburn results.

#### NITROGEN AVAILABILITY AND PLANT GROWTH.

The intimate relationship between the extent of loss of nitrate during winter leaching and the reduction in final crop yield or nitrogen recovery in these experiments is shown in Fig. 7 and in the following correlation coefficients and regression equations:

$a$  = nitrate nitrogen in total leachate in mg. per pot.

$b$  = nitrogen content of unleached crop less nitrogen content of leached crop in mg. per pot.

$c$  = dry weight of grain + straw in unleached crop less dry weight of grain and straw in leached crop in gm. per pot.

$$r_{ba} = +0.87; [b = 1.89(a - 44.5): a = 0.40b + 63];$$

$$r_{ca} = +0.92; [c = 0.267(a - 44.2): a = 3.15c + 50].$$

The correlation coefficients are highly significant and the regression lines bring out the important result that the adverse effect of leaching

on the final plant is not proportional to the amount of nitrate removed. About 50 mg. of nitrogen per pot may be removed without reducing the crop, but as the nitrogen loss increases above this amount the nitrogen content of the plant falls off about twice as rapidly. This is clear proof that early nitrification and leaching lead to an indirect loss of available nitrogen which is much greater than the actual amount of

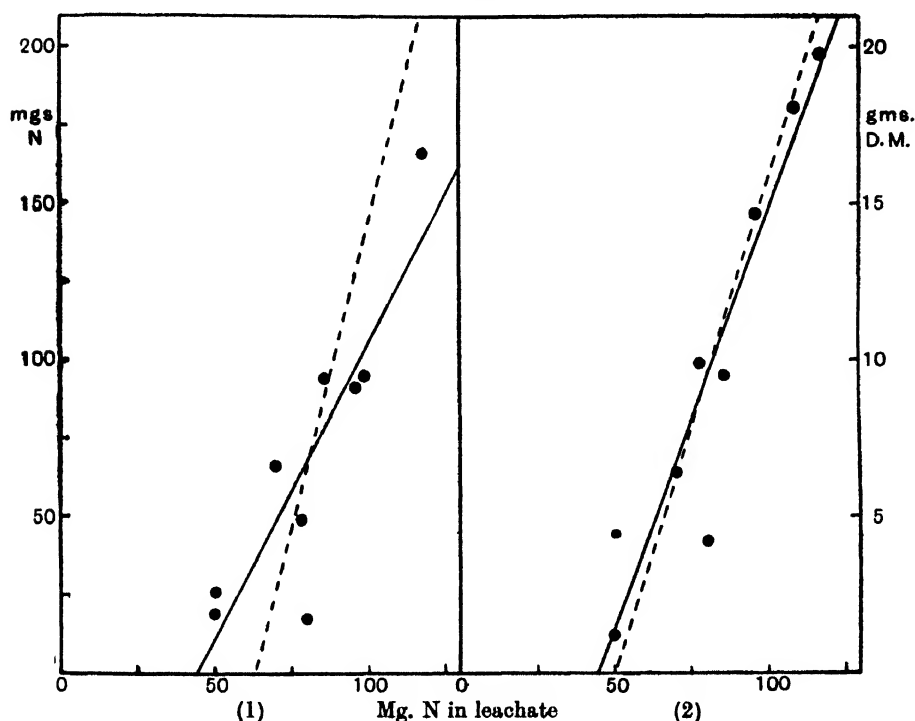


Fig. 7. Relationship of the nitrate in leachate and the reduction by leaching of (1) nitrogen content of grain + straw, and (2) dry weight of grain + straw.

extra nitrate lost. For spring applications of sodium nitrate or ammonium sulphate the recovery of nitrogen by wheat rarely exceeds one-third and for autumn applications is generally still lower. Nitrate produced from green manure residues thus behaves quite differently from nitrate from artificial fertilisers.

Although the present series of experiments was devised to measure the extent and effect of the loss rather than to throw light on its mechanism, it is appropriate to refer here to one or two of the hypotheses now being examined in extending this work. The better root systems of the young plants in the soils with low initial nitrate contents may enable them to utilise later supplies more effectively. The well-established





but unexplained loss of total nitrogen from cereal soils may be greater in soils producing appreciable amounts of nitrate in autumn and early winter. We are of opinion, however, that the principal factor is the influence of the added organic matter and the leaching on the competition between the plant and the soil micro-organisms at different stages during the growth of the plant. The three untreated soils used in our experiments contained relatively large amounts of available nitrogen and relatively little available carbon compounds, for there had been sufficient opportunity during three months fallow in a dry spell to allow oxidation without leaching. The yields in the unleached soils showed that the reserves of available nitrogen increased in the order fallow plot, mustard plot, tares plot, as would be expected. Leaching reduced this immediately available nitrogen, and in the richer soils the leached plants had less nitrogen than the unleached, even during the period of leaching. The low nitrate content of the soil would retard further oxidation of the carbon compounds of the soil sufficiently to allow it to continue in spring and early summer and so cause serious competition for nitrogen between the plant and the micro-organism during the critical period of active growth of the wheat.

When tares were added to unleached soil there would be a considerable liberation of ammonia and nitrate, but much of the nitrogen would be locked up again in the decomposition of carbon from the tares and from the soil. This decomposition would proceed rapidly at first in the presence of much readily available nitrogen, but later, when most of the carbon compounds were oxidised and the plant was able to assimilate large amounts of nitrate, competition with the micro-organism would be reduced. Leaching the soil treated with tares reduced the nitrate concentration, but the liberation of ammonia and nitrate was sufficiently rapid to meet the small requirements of the young plant, for there was no appreciable effect of leaching on the nitrogen content of the young plants at the time of thinning. The decomposition of carbon compounds would, however, be retarded by the removal of nitrate sufficiently to increase the assimilating power of the micro-organisms during the critical period of rapid nitrogen uptake by the wheat. Although some of the micro-organic proteins would ultimately yield nitrogen available for the plants, much of it would be too late to be fully utilised for growth and the total reduction by leaching would be greater than the amount of nitrate actually found in the drainage water.

Mustard provided insufficient nitrogen for the decomposition of the carbon compounds added, and the immediate supply of ammonia and

nitrate was greatly reduced for a long period. Ultimately when the excess carbon had been oxidised more nitrogen would be provided for the plant. It appears that in the present experiments the extra late nitrogen just counterbalanced the effect of the initial shortage of nitrogen. For low initial nitrate contents leaching could have little effect on nitrate assimilation by the plant or by the micro-organisms.

The plant development curves show very clearly the progressive decline of plants in the leached tares series and the steady advance of those in both the mustard series relative to the general means. Although the leached tares treatment did not prove significantly poorer than the leached mustard series, there are indications that this result might have been obtained if the experiment had been commenced earlier to provide greater opportunity for nitrification and leaching before the wheat was sown. In the present experiment nitrification and growth proceeded for 6 weeks before the leaching was commenced. Although the soil from the tares-plot soil was richer than the fallow-plot soil treated with tares under unleached conditions, it suffered so much more from leaching that it gave a lower yield and nitrogen recovery in the leached series. Although there was less initial available nitrogen in the fallow-plot soil with tares, the fresh organic matter was able to retain more nitrogen for the critical early summer period. Additional support for this explanation of the discrepancy between the pot experiments and the usual field results is provided by the exceptional results of 1928-9 in the Woburn field experiments. In Lansome field the wheat followed immediately after the ploughing in of the green manures, and the winter was cold and dry. The yields were exceptional in that they approached those of wheat in commercial cropping and tares gave better wheat than mustard. The conditions and the results resemble those of our unleached experiments. But in Stackyard field in the same season there was a 3 months' interval with much rain between folding of the green manures and drilling the wheat. The yields after both tares and mustard were extremely low and the tares plot gave the lower yield. The conditions resemble those in our leached-tares and mustard-plot soils which gave the lowest yields of the whole experiment. Our series with leaching after tares and mustard additions fell between the conditions of the two 1928-9 field experiments and tares proved about equal to mustard but better than no addition.

Loss of nitrate by leaching from light land is not confined to the winter months and in a two-course green manure-wheat rotation may be considerable during the 9 months of the green manure shift in which

the soil is either bare or carrying a young crop as well as in the 5 winter months in which the ground is occupied by young wheat. Although the tares plot receives nitrogen from the air and accumulates some of it as humus, and although the mustard plot carries over nitrogen from the autumn to the end of the following summer or later, there is still ample opportunity for the loss of the extra available nitrogen before the next wheat crop is able to use it. Further, such losses should become progressively worse, for they are accompanied by rapid oxidation of carbon compounds and so decrease the ability of the soil to resist subsequent leaching. We suggest therefore that the lack of adjustment between the time of producing and the time of utilising the soluble nitrogen in such rotations is the principal factor in the exhaustion of the Woburn green manure plots.

Better ways of utilising the characteristic effects of tares and mustard on light soils in mild wet climates may be suggested. Where tares can be grown successfully as an autumn-sown crop to stand the winter it will provide an abundant supply of readily available nitrogen for crops sown in spring shortly after ploughing in either the whole crop or its residues after folding. It is, however, essential that the tares crop should be sufficiently far advanced by early winter to prevent much loss of nitrate in drainage. A poor tares crop wastes available nitrogen; only a good one can add available nitrogen. The nitrogen of tares must be utilised almost as if it were given as an artificial fertiliser; tares crops in spring or early summer should be followed by rapidly growing crops such as roots, cabbage, kale or other fodder crops<sup>1</sup>. Late summer crops may be wasted if followed by late sown and slow-growing winter cereals on light freely draining soil, but appreciable amounts of the nitrogen may be utilised and conserved by winter rye or barley which may be sown earlier and grow more rapidly. Experiments are now in progress to ascertain whether the incorporation of straw before sowing wheat after tares can also be used to carry over available nitrogen from autumn to summer.

Mustard should be regarded as a means of locking up nitrogen and liberating it again some months later. It therefore forms a useful catch crop for summer and autumn. Again it is necessary to have good crops; starved ones provide too great an opportunity for loss of nitrate by

<sup>1</sup> Compare Arthur Young, *The Farmer's Calendar*, 10th edition, 1815, 458: "A good crop of winter tares leaves the ground in such loose, putrid, friable order, that it is better husbandry to sow turnips or plant cabbage on it, than to leave it to receive tillage for wheat."



drainage, especially when they are grown frequently on the same land.

These suggestions are applicable only to light soils in regions in which the climatic conditions allow abundant drainage at all times. In heavier soils or with lower rainfall or lower winter temperatures drainage is less and the differences between tares and mustard are less marked. With heavier soils and drier climates the physical effects of the organic matter, both fresh and humified, become more important. Under such conditions the actual additions to the soil, carbon for all green manures and nitrogen as well for leguminous ones, may become the primary factors and the composition of the green manures only secondary.

#### DISCUSSION.

Although we are not as yet in a position to extend this interpretation of the action of green manures by actual measurements of the amounts of readily oxidisable carbon and nitrogen under conditions sufficiently similar to those in the field, we would suggest that the conception of a labile equilibrium between them and consideration of the effects of displacements in this equilibrium on the time at which soluble nitrogen is produced will facilitate the interpretation of a number of light land problems relating to residual effects of fertilisers, organic manures and other crops on succeeding crops. The "humus content" of the soil is of little importance in this connection, for the organic materials which are readily extracted or oxidised *in vitro* are almost inert in the biochemical changes involved in the production and assimilation of available nitrogen. Until it becomes possible to eliminate the nitrifying organisms by differential sterilisation, it appears necessary to maintain such ratios of available carbon to available nitrogen as will reduce nitrate production to a minimum except when there is an actively growing crop to take it up. Further, that ill-defined "condition" or "good heart" which is so highly prized by the farmer may depend on having sufficient reserves of available carbon and nitrogen in the soil to "buffer" it against too violent fluctuations in their ratio on the addition of more organic matter or fertilisers or on the removal of ammonia and nitrates by the plant or by leaching. The "slow and steady" action of nitrogenous fertilisers desired by farmers probably depends more on the previous history of the soil than on the specific properties of the fertiliser.

The Norfolk four-course rotation (roots, barley, seeds, wheat) provides an excellent illustration of the adjustment of the additions of carbon and nitrogen compounds to provide nitrogen to the crops at the

best times. In only one autumn in four is the soil cultivated for a slowly growing winter crop (wheat), and it is recognised that after this the soil needs heavy dressings of dung to restore its fertility. In the other autumns there is either an actively growing crop (roots and seeds ley) or the residues of a stubble rich in carbon. Fear of soil exhaustion was the principal ground for the former restrictions on cropping which prevented the taking of consecutive corn crops, *i.e.* of leaving the soil either bare or with a very small crop through two winters. This method of reducing the buffering with readily decomposable carbon and nitrogen compounds is, however, deliberately adopted when it is desired to have an early flush of nitrate with little late nitrate for barley intended for malting. Fallowing reduces the available carbon compounds and gives a temporary accumulation of available nitrogen and therefore a better crop immediately following the fallow, but the gain is at the expense of the general level of soil fertility. It may be suggested, too, that the merit of "sheeping" lies partly in maintaining a growing crop well into the winter and partly in compacting the soil and thus reducing the rate of percolation and providing more opportunity for the assimilation of soluble nitrogen compounds by the soil organisms.

The art of maintaining the fertility of light land in wet temperate regions involves in addition to supplying adequate amounts of lime, phosphoric acid, and potash, the maintenance of sufficient reserves of decomposable organic matter, with such an adjustment of the balance between available nitrogen and carbon as will lock up nitrogen in insoluble forms except when it is required by an actively growing crop. This balance may be secured by adding organic matter of relatively high C : N ratio in the autumn (*e.g.* cereal stubbles, straw, sugar beet tops), by having actively growing crops in the autumn and early winter (*e.g.* temporary leys, trefoil, roots, kale, rye, or even weeds), and by adding both nitrogen and carbon by leguminous plants in leys. Experiments are being undertaken at Woburn to test these possibilities.

#### SUMMARY.

1. It is suggested that the striking failure of winter wheat grown in rotation with two summer crops of tares or mustard on the sandy soil of the Woburn Experimental Station is due to the production of nitrate and ammonia from the green manures at times when the wheat is unable to use them efficiently and to the consequent loss of nitrate in the drainage. Owing to its low C : N ratio the nitrogen in tares nitrifies very rapidly and the loss by leaching is very great. Mustard, on the other

hand, reduces the winter loss, but the nitrogen present in the mustard and that absorbed in the decomposition of the excess carbon compounds are liberated too slowly to be utilised efficiently by the wheat and much of the nitrate subsequently produced is also lost by leaching.

2. Nitrification experiments in the laboratory and pot experiments on wheat showed that nitrogen was made available more rapidly and more completely from materials with 13C : 1N (tares, mustard + blood, straw + blood) than from those with 26C : 1N (tares + straw, mustard, straw + blood). The yields in unleached pots were much higher with materials with 13C : 1N or in untreated soil, but in pots leached systematically during the winter the two types of organic matter were equally effective in increasing the yield. The reduction of crop by leaching was closely correlated with, but not proportional to, the extent of early nitrate formation as measured by the amount of nitrate leached from the pots. It is suggested that early nitrate formation reduces the yield not only by increasing the removal of nitrate by leaching but also by increasing the amount converted by the soil micro-organisms into forms which become available again only very slowly.

3. Tares material formed nitrates and mustard material removed it more rapidly and completely than equivalent mixtures. Under all conditions tested tares material proved rather less effective for grain production than the other materials. The less intimate association of the proteins and cellulosic substances in the mixtures appears to be sufficient explanation of these differences. There was no evidence of specific toxics or stimulants in mustard or tares.

#### ACKNOWLEDGMENTS.

We wish to record our indebtedness to Dr H. H. Mann for advice and assistance, especially in arranging and conducting the pot experiments and analyses at the Woburn Experimental Station, and to Dr J. Wishart for help in the application of the Analysis of Variance to our data.

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**On the frequency distribution of the means of samples  
from populations of certain of Pearson's types**

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## INTRODUCTORY

(i) The *exact* distributions of statistical estimates made from samples of non-normal populations are almost entirely unknown.

The distribution of the means of samples from normal populations dates back, of course, to GAUSS or even before, but it is to the powerful geometrical methods of FISCHER that we owe a precise demonstration of the distribution of the variance, (1), (first obtained by STUDENT's intuition, (2)), the distribution of the correlation coefficient, (1), of the regression coefficient, (3), of the correlation ratio, (3), of partial, (4), and finally of multiple correlation coefficients (5). Work on these distributions has also been done by PEARSON (6), by ROMANOVSKY (7) and by WISHART (8) The exact distribution of the higher moments is at present unsolved for the normal case.

Some writers, conscious of the difficulties, still for the most part unsurmounted, of obtaining exact solutions for non-normal populations have concentrated their attention on obtaining *the moments* of such sampling distributions, PEARSON for instance has given many approximate formulae for the second moment of moment coefficients (9) while TCHOUPROFF was the first to give exact results for the moments of the mean and the first four moments of the variance (10). A full treatment of this problem of obtaining the moments of any moment function has recently been given by FISHER (11) while WISHART (12) has shown how to apply FISHER's results to normal populations.

Now that the moments of moment coefficients can be obtained whatever the nature of the population sampled, the most promising way of obtaining their exact distributions seems to be by way of the characteristic function.

Thus if  $\varphi_r(x) dx$  gives the sampling distribution of the  $r^{\text{th}}$  moment coefficient  $m_r$ , whose moments are  $\mu(r^1) \mu(r^2) \dots \mu(r^n) \dots$  we have

$$\int_{-\infty}^{\infty} \varphi_r(x) dx = 1.$$

$$\int_{-\infty}^{\infty} e^{ax} \varphi_r(x) dx = \sum_{s=0}^{\infty} \mu(r^s) \frac{a^s}{s!} = \psi_r(a)$$

say if we write  $\alpha = i \beta$

$$\int_{-\infty}^{\infty} e^{i\beta x} \varphi_r(x) dx = \psi_r(i\beta)$$

and hence in general, by FOURIER'S Integral Theorem

$$\psi_r(x) = \frac{1}{2\pi} \int_{-\infty}^{\infty} e^{-i\beta x} \varphi_r(i\beta) d\beta$$

Such functions as  $\psi_r(i\beta)$  are known as characteristic functions and their theory has been discussed rigorously by PAUL LEVY (13).

The present writer independently applied a similar method (14) to the determination of the frequency distribution of the mean and discussed as particular cases the distributions of the means of samples from populations of PEARSON'S Type III and Type II whose standard forms are

$$\text{Type III} \quad y = y_0 e^{-\frac{p}{a}} \left(1 + \frac{x}{a}\right)^p$$

$$\text{Type II} \quad y = y_0 \left(1 - \frac{x^2}{a^2}\right)^m$$

The object of the present paper is to determine the precise distributions of the means of samples from Populations of PEARSON'S Type I and Type VII whose standard forms are

$$\text{Type I} \quad y = y_0 \left(1 + \frac{x}{a_1}\right)^{m_1} \left(1 - \frac{x}{a_2}\right)^{m_2}$$

$$\text{Type VII} \quad y = y_0 \left(1 + \frac{x^2}{a_2}\right)^{-m}$$

In what follows the forms

$$\text{Type I} \quad y = c x^{p-1} (1-x)^{q-1}$$

$$\text{Type VII} \quad y = c (1+x^2)^{-m}$$

will be used. These are easily reduced from the standard forms and more easily handled analytically. The Pearsonian system of frequency distributions forms a widely known system so that the results may be of interest both in themselves and as illustrating the general method.

(ii) In the previous paper (14), it was shown that if  $y = f(x)$  be any frequency distribution and if samples of size  $n$  be drawn at random from a population of this type, supposed indefinitely large, then, if  $Y = \psi(x)$  is the frequency distribution of totals (i. e.  $n$  times the mean) in such samples,

$$\left( \int_a^b f(x) e^{\alpha x} dx \right)^n = \int_{na}^{nb} \psi(x) e^{\alpha x} dx \quad (1)$$

$a$  to  $b$  being the range of the original frequency distribution. This led to the solution

$$\psi(x) = \frac{1}{2\pi} \int_{-\infty}^{\infty} e^{-i\beta x} F(i\beta) d\beta, \quad na \leq x \leq nb \quad (2)$$

where

$$F(\alpha) = \left( \int_a^b f(x) e^{\alpha x} dx \right)^n$$

provided  $\int_{-\infty}^{\infty} e^{-i\beta x} F(i\beta) d\beta = 0$  where  $x < na$  or  $> nb$

From (2) by the simple substitution  $x = n\bar{x}$  the distribution of means was at once obtained

This method was applied to the normal curve, to Pearson's Type III and to PEARSON'S Type II

$$y = x^{p-1} (1-x)^{p-1}$$

the distribution of means of the latter case being

$$y = \frac{n}{2} (\sqrt{\pi})^{n-2} \{\Gamma(p)\}^n \int_{-\infty}^{\infty} \left\{ J_{p-\frac{1}{2}} \left( \frac{\beta}{2} \right) \right\}^n \cos(n\bar{x}\beta) d\beta \quad (3)$$

By putting  $p = 1$ , the case of a rectangular distribution was reached and discussed in some detail, and it was pointed out that, for all integral values of  $p$  the function under the integral sign in (3) reduces to a trigonometrical function which is integrable.

Subsequently the writer performed the integrations and deduced explicitly the frequency distribution of the mean in the case  $p = 2$  for all values of  $n$  (the size of sample), and for  $p = 3$ ,  $p = 4$  for samples of 2, 3 and 4. Still later however he realised that it was pos-



sible to obtain, by the same method, the frequency distribution of the mean in samples from the more general Type I

$$y = c x^{p-1} (1-x)^{q-1}$$

for integral values of  $p$  and  $q$  and that the distributions which he had deduced from (3) were merely particular cases, when  $p = q$ , of the distributions arising from Type I populations. Accordingly the more general case will now be considered.

## PART I.

THE DISTRIBUTION OF THE MEANS OF SAMPLES FROM PEARSON'S  
TYPE I FOR INTEGRAL VALUES OF  $p$  AND  $q$ .

### (A) *The General Solution.*

The Type I curve may be written

$$y = \frac{\Gamma(p+q)}{\Gamma(p)\Gamma(q)} x^{p-1} (1-x)^{q-1}$$

hence if

$$F(x) = \left\{ \int_0^x \frac{\Gamma(p+q)}{\Gamma(p)\Gamma(q)} x^{p-1} (1-x)^{q-1} e^{\alpha x} dx \right\}^n \quad (4)$$

the distribution of totals is given by

$$\psi(x) = \frac{1}{2\pi} \int_{-\infty}^{\infty} e^{-i\beta x} F(i\beta) d\beta \quad 0 \leq x \leq n \quad (5)$$

provided this integral is zero when  $x$  is greater than  $n$  or negative.

Consider the expression  $F(x)$ . It is known that if  $M_{k,m}(z)$  is the confluent hypergeometric function (15) defined by

$$M_{k,m}(z) = z^{\frac{1}{2}+m} e^{-\frac{1}{2}z} \left\{ 1 + \frac{\frac{1}{2}+m-k}{1!(2m+1)} z + \right. \\ \left. + \frac{\left(\frac{1}{2}+m-k\right)\left(\frac{3}{2}+m-k\right)}{2!(2m+1)(2m+2)} z^2 + \dots \right\}$$

then

$$M_{k,m}(z) = C \int_{-1}^1 (1+u)^{-\frac{1}{2}+m-k} (1-u)^{-\frac{1}{2}+m+k} e^{\frac{1}{2}zu} du$$

where

$$C = \frac{2^{-2m} \Gamma(2m+1) z^{m+\frac{1}{2}}}{\Gamma\left(\frac{1}{2}+m+k\right) \Gamma\left(\frac{1}{2}+m-k\right)}$$

On writing  $1-u=2w$  we find

$$M_{k,m}(z) = 2^{2m} C e^{\frac{1}{2}z} \int_0^1 e^{-zw} (1-w)^{-\frac{1}{2}+m-k} w^{-\frac{1}{2}+m+k} dw$$

Now put

$$p-1 = -\frac{1}{2} + m + k$$

$$q-1 = -\frac{1}{2} + m - k$$

we find

$$m = \frac{1}{2}(p+q-1)$$

$$k = \frac{1}{2}(p-q)$$

whence

$$\begin{aligned} M_{\frac{1}{2}(p-q), \frac{1}{2}(p+q-1)}(-\alpha) &= \\ &= e^{-\frac{\alpha}{2}} \frac{\Gamma(p+q)}{\Gamma(p) \Gamma(q)} (-\alpha)^{\frac{1}{2}(p+q)} \int_0^1 e^{\alpha x} x^{p-1} (1-x)^{q-1} dx \end{aligned}$$

and

$$\begin{aligned} &\int_0^1 e^{\alpha x} x^{p-1} (1-x)^{q-1} dx = \\ &= \frac{\Gamma(p) \Gamma(q)}{\Gamma(p+q)} e^{\frac{\alpha}{2}} (-\alpha)^{-\frac{1}{2}(p+q)} M_{\frac{1}{2}(p-q), \frac{1}{2}(p+q-1)}(-\alpha) \end{aligned}$$

Whence

$$F(\alpha) = e^{n\frac{\alpha}{2}} \left[ \frac{M_{\frac{1}{2}(p-q), \frac{1}{2}(p+q-1)}(-\alpha)}{(-\alpha)^{\frac{1}{2}(p+q)}} \right]^n \quad (*) \quad \dots\dots (6)$$

(\*) It is interesting to see how this reduces to the known case of Type II when  $p = q$ .

In this case we have

$$\int_0^1 e^{\alpha x} x^{p-1} (1-x)^{p-1} dx = e^{\frac{\alpha}{2}} \left[ \frac{M_{0, p-\frac{1}{2}}(-\alpha)}{(-\alpha)^p} \right]$$

and  $M_{0, p-\frac{1}{2}}(-\alpha)$  may be written (see (16) p. 332).

$$M_{0, p-\frac{1}{2}}(-\alpha) = (-\alpha)^p \left\{ 1 + \sum_{s=1}^{\infty} \frac{\alpha^{2s}}{2^{4s} s! \left(p + \frac{1}{2}\right) \left(p + \frac{3}{2}\right) \dots \left(p + s - \frac{1}{2}\right)} \right\}$$

whence

$$\begin{aligned} \frac{M_{0, p-\frac{1}{2}}(-\alpha)}{(-\alpha)^p} &= 1 + \frac{(\alpha/2)^2}{2^2 1! \left(p + \frac{1}{2}\right)} + \frac{(\alpha/2)^4}{2^4 2! \left(p + \frac{1}{2}\right) \left(p + \frac{3}{2}\right)} + \\ &\dots\dots = 2^{p-\frac{1}{2}} \Gamma\left(p + \frac{1}{2}\right) \frac{I_{p-\frac{1}{2}}\left(\frac{\alpha}{2}\right)}{\left(\frac{\alpha}{2}\right)^{p-\frac{1}{2}}} \end{aligned}$$

where  $I_n(x)$  is BESSEL'S Function of order  $n$  with imaginary argument.

$$\begin{aligned} \text{Hence } F(\alpha) &= \left[ e^{\frac{\alpha}{2}} 2^{2p-1} \Gamma\left(p + \frac{1}{2}\right) \frac{I_{p-\frac{1}{2}}\left(\frac{\alpha}{2}\right)}{\alpha^{p-\frac{1}{2}}} \right]^n \\ &= \left[ \frac{2^{2p-1} \Gamma\left(p + \frac{1}{2}\right)}{\Gamma(p) \Gamma\left(\frac{1}{2}\right)} \left\{ e^{\frac{\alpha}{2}} \Gamma(p) \Gamma\left(\frac{1}{2}\right) \alpha^{-p+\frac{1}{2}} I_{p-\frac{1}{2}}\left(\frac{\alpha}{2}\right) \right\} \right]^n \\ &= \left[ \frac{\Gamma(2p)}{\Gamma(p) \Gamma(p)} \left\{ e^{\frac{\alpha}{2}} \Gamma(p) \Gamma\left(\frac{1}{2}\right) \alpha^{-p+\frac{1}{2}} I_{p-\frac{1}{2}}\left(\frac{\alpha}{2}\right) \right\} \right]^n \end{aligned}$$

as obtained in the previous paper (14, p. 230).

Thus the distribution of totals will be given by

$$\psi(x) = \frac{1}{2\pi} \int_{-\infty}^{\infty} e^{-i\beta x} e^{\frac{n+1}{2}\beta} \left\{ \frac{M^{\frac{1}{2}(p-q), \frac{1}{2}(p+q-1)}(-i\beta)^n}{(-i\beta)^{\frac{1}{2}(p+q)}} \right\} d\beta$$

and the distribution of means by

$$y = \frac{n}{2\pi} \int_{-\infty}^{\infty} e^{-in\beta(\bar{x} - \frac{1}{2})} \left\{ \frac{M^{\frac{1}{2}(p+q), \frac{1}{2}(p+q-1)}(-i\beta)^n}{(-i\beta)^{\frac{1}{2}(p+q)}} \right\} d\beta$$

or on transferring the origin to the point  $\bar{x} = \frac{1}{2}$

$$y = \frac{n}{2\pi} \int_{-\infty}^{\infty} e^{-in\beta\bar{x}} \left\{ \frac{M^{\frac{1}{2}(p-q), \frac{1}{2}(p+q-1)}(-i\beta)^n}{(-i\beta)^{\frac{1}{2}(p+q)}} \right\} d\beta$$

where  $-\frac{1}{2} < x \leq \frac{1}{2}$  ..... (7)

In order that this solution may be valid it is necessary to show that the integral (7) is zero when  $x > \frac{1}{2}$  or  $x < -\frac{1}{2}$ . This may be done by contour integration. (See footnote to p. 11)

We will now show that (7) is actually integrable when  $p, q$  are integers.

It is known that when

$$-\frac{1}{2}\pi < \arg z < \frac{3}{2}\pi \text{ and } -\frac{3}{2}\pi < \arg(-z) < \frac{1}{2}\pi$$

$$M_{k,m}(z) = \frac{\Gamma(2m+1)}{\Gamma\left(\frac{1}{2}+m-k\right)} e^{k\pi i} W_{-k,m}(-z) +$$

$$+ \frac{\Gamma(2m+1)}{\Gamma\left(\frac{1}{2}+m+k\right)} e^{\left(\frac{1}{2}+m+k\right)\pi i} W_{k,m}(z)$$

where  $W_{k,m}(z)$  is WHITTAKER'S confluent hypergeometric function (16, p. 340). This gives

$$M_{\frac{1}{2}(p-q), \frac{1}{2}(q+p-1)}(z) = \frac{\Gamma(p+q)}{\Gamma(q)} e^{\frac{1}{2}(p-q)\pi i} W_{-\frac{1}{2}(p-q), \frac{1}{2}(p+q-1)}(-z) \\ + \frac{\Gamma(p+q)}{\Gamma(p)} e^{p\pi i} W_{\frac{1}{2}(p-q), \frac{1}{2}(p+q-1)}(z)$$

where if  $z$  is real and positive we must take  $\arg z = 0$ ,  $\arg(-z) = -\pi$ ; if  $z$  is real and negative we must take  $\arg(z) = \pi$ ,  $\arg(-z) = 0$ . Hence with this convention

$$M_{\frac{1}{2}(p-q), \frac{1}{2}(p+q-1)}(-\alpha) = \frac{\Gamma(p+q)}{\Gamma(q)} e^{\frac{1}{2}(p-q)\pi i} W_{-\frac{1}{2}(p-q), \frac{1}{2}(p+q-1)}(\alpha) \\ + \frac{\Gamma(p+q)}{\Gamma(p)} e^{p\pi i} W_{\frac{1}{2}(p-q), \frac{1}{2}(p+q-1)}(-\alpha) \\ \dots\dots (8)$$

Now using the asymptotic expansion for the Whittaker functions, we have

$$W_{\frac{1}{2}(p-q), \frac{1}{2}(p+q-1)}(z) \sim e^{-\frac{1}{2}z} z^{\frac{1}{2}(p-q)} \left\{ 1 + \sum_{s=1}^{\infty} \frac{A_s}{s! z^s} \right\}$$

where

$$A_s = \left\{ \frac{1}{4} (p+q-1)^2 - \frac{1}{4} (p-q-1)^2 \right\} \\ \times \left\{ \frac{1}{4} (p+q-1)^2 - \frac{1}{4} (p-q-3)^2 \right\} \dots\dots \\ \dots\dots \left\{ \frac{1}{4} (p+q-1)^2 - \frac{1}{4} (p-q-2s-1)^2 \right\}$$

and

$$W_{-\frac{1}{2}(p-q), \frac{1}{2}(p+q-1)}(z) \sim e^{-\frac{1}{2}z} z^{\frac{1}{2}(q-p)} \left\{ 1 + \sum_{s=1}^{\infty} \frac{B_s}{s! z^s} \right\}$$

where  $A_s, B_s$  may also be written

$$A_s = (p-1)(p-2)\dots(p-s)q(q+1)\dots(q+s-1)$$

$$B_s = (q-1)(q-2)\dots(q-s)p(p+1)\dots(p+s-1)$$

$$\sim (-1)^p e^{-i n \beta \bar{x}} \left\{ \Gamma(p+q) \right\}^n \sum_{s=0}^n \left\{ \Gamma(q) \right\}^s \left\{ \Gamma(p) \right\}^{n-s} (i\beta)^{ps+q(n-s)}$$

$$\left| \varphi(\beta) \right| \sim \left| \sum_{s=0}^n Q_s \frac{e^{-i n \beta \left( \bar{x} - \frac{1}{2} + \frac{s}{n} \right)}}{(i\beta)^{ps+q(n-s)}} \right|$$

where 
$$Q_s = \frac{(-1)^p \left\{ \Gamma(p+q) \right\}^n}{\left\{ \Gamma(q) \right\}^s \left\{ \Gamma(p) \right\}^{n-s}}$$

Now suppose  $\bar{x} < -\frac{1}{2}$

Then since  $s$  can only take the values  $0, 1, 2, \dots, n$ ,  $\bar{x} - \frac{1}{2} + \frac{s}{n}$  is negative for all values of  $s$  say  $= -\mu_s$

and 
$$\left| \frac{e^{-i n \beta \left( \bar{x} - \frac{1}{2} + \frac{s}{n} \right)}}{(i\beta)^{ps+q(n-s)}} \right| = \frac{e^{-n\mu_s r \sin \theta}}{|\beta|^{ps+q(n-s)}}$$

Hence 
$$\left| \varphi(\beta) \right| < A e^{-k r \sin \theta} \quad k > 0$$

where  $A$  is independent of  $\beta$

Hence 
$$\int_{BCA} \varphi(\beta) d\beta \rightarrow 0 \text{ as } |\beta| \rightarrow \infty$$

and 
$$\int_{-\infty}^{\infty} \varphi(\beta) d\beta = 0$$

If  $\bar{x} > \frac{1}{2}$ ,  $\bar{x} - \frac{1}{2} + \frac{s}{n}$  is positive for all values of  $s$  and the same result follows by taking a semi-circular contour below the real axis.

But if  $-\frac{1}{2} < \bar{x} < \frac{1}{2}$

$\bar{x} - \frac{1}{2} + \frac{s}{n}$  is positive for some values of  $s$  and negative for others and  $\int \varphi(\beta) d\beta$  does not vanish round either semi-circular contour, and the integral is of course not zero.

Now from (7) we see that the distribution may be written

$$y = \frac{n}{2\pi} \int_{-\infty}^{\infty} G(\beta) d\beta$$

where

$$G(\beta) = e^{-in\beta x} \left\{ \frac{M_{\frac{1}{2}(p-q), \frac{1}{2}(p+q-1)}^{(\frac{1}{2}\beta)} (-\beta)^n}{-(\frac{1}{2}\beta)^{\frac{1}{2}(p+q)}} \right\}$$

or

$$y = \frac{n}{2\pi} \lim_{\epsilon \rightarrow 0} \left[ \int_{-\infty}^{-\epsilon} G(\beta) d\beta + \int_{-\epsilon}^{\infty} G(\beta) d\beta \right] \quad \dots (11a)$$

Now the integrand has no pole at  $\beta = 0$ , therefore the LAURENT expansion of (11a) in powers of  $\epsilon$ , must be such that all the coefficients of negative powers of  $\epsilon$  vanish.

Now on substituting the value of  $G(\beta)$  given by (11) in

$$\int_{-\infty}^{-\epsilon} G(\beta) d\beta + \int_{\epsilon}^{\infty} G(\beta) d\beta$$

and expanding the multinomial the expression is seen, when  $p$  and  $q$  are integers, to consist of a finite number of terms of the type

$$C \left( \int_{-\infty}^{-\epsilon} + \int_{\epsilon}^{\infty} \right) e^{-in\beta x} e^{\frac{1}{2}i\beta(n-2r)} \left( \frac{1}{i\beta} \right)^s d\beta$$

(where  $r$  is an integer and  $0 \leq r < n$ )

$$= C \left( \int_{-\infty}^{-\epsilon} + \int_{\epsilon}^{\infty} \right) e^{-in\beta \left( x - \frac{1}{2} + \frac{r}{n} \right)} \left( \frac{1}{i\beta} \right)^s d\beta$$

But

$$\left( \int_{-\infty}^{-\epsilon} + \int_{\epsilon}^{\infty} \right) \frac{e^{-in\beta \left( x - \frac{1}{2} + \frac{r}{n} \right)} d\beta}{(i\beta)^s} = i^{-s} \left( \int_{-\infty}^{-\epsilon} + \int_{\epsilon}^{\infty} \right) \left( \frac{\cos nc\beta - i \sin nc\beta}{\beta^s} \right) d\beta$$

Now if  $s$  is even

$$\int_{\epsilon}^{\infty} \frac{\sin nc\beta}{\beta^s} d\beta = - \int_{-\infty}^{-\epsilon} \frac{\sin nc\beta}{\beta^s} d\beta$$

Hence

$$\begin{aligned} \left( \int_{-\infty}^{-\epsilon} + \int_{\epsilon}^{\infty} \right) \frac{e^{-i nc\beta}}{(i\beta)^s} d\beta &= 2i^{-s} \int_{\epsilon}^{\infty} \frac{\cos nc\beta}{\beta^s} d\beta \\ &= 2(-1)^{\frac{s}{2}} \sum_{p=0}^{s-2} (nc)^p \cos\left(nc\epsilon + \frac{p\pi}{2}\right) \epsilon^{s-p-1} \\ &\quad + \frac{2(nc)^{s-1}}{(s-1)!} \int_{\epsilon}^{\infty} \frac{\sin nc\beta}{\beta} d\beta \quad \dots (11b i) \end{aligned}$$

But if  $s$  is odd

$$\int_{-\infty}^{-\epsilon} \frac{\cos nc\beta}{\beta^s} d\beta = - \int_{\epsilon}^{\infty} \frac{\cos nc\beta}{\beta^s} d\beta,$$

and

$$\begin{aligned} \left( \int_{-\infty}^{-\epsilon} + \int_{\epsilon}^{\infty} \right) \frac{e^{-i nc\beta}}{(i\beta)^s} d\beta &= -2i^{-s+1} \int_{\epsilon}^{\infty} \frac{\sin nc\beta}{\beta^s} d\beta \\ &= 2(-1)^{\frac{s+1}{2}} \sum_{p=0}^{s-2} (nc)^p \sin\left(nc\epsilon + \frac{p\pi}{2}\right) \epsilon^{s-p-1} \\ &\quad - \frac{2(nc)^{s-1}}{(s-1)!} \int_{\epsilon}^{\infty} \frac{\sin nc\beta}{\beta} d\beta \quad \dots (11b ii) \end{aligned}$$

Thus a whole series of expressions of the type (11bi) and (11bii) have to be substituted in (11a) and when this is done we obtain the LAURENT expansion of 11a in positive and negative powers of  $\epsilon$ . The coeffi-



cients of all the negative powers must as we have seen vanish, further no contribution is made to the term independent of  $\varepsilon$  by the series in (11b), because each series consists of cosines divided by odd powers of  $\varepsilon$  and sines divided by even powers of  $\varepsilon$ . Thus these series contribute only to the positive powers of  $\varepsilon$  in the LAURENT expansion and these vanish in the limit.

Thus only the integrals in (11b) need be taken into account in evaluating 11a and therefore (7).

I propose to write this result symbolically:—

$$\begin{aligned} \text{cont.} \int_{-\infty}^{\infty} \frac{e^{-i n c \beta} d\beta}{(i\beta)^s} &= \frac{2(n c)^{s-1}}{(s-1)!} \int_0^{\infty} \frac{\sin n c \beta}{\beta} d\beta \\ &= \frac{|n c|^{s-1} \pi}{(s-1)!} \quad \text{if } s \text{ is even,} \end{aligned}$$

and

$$\text{cont.} \int_{-\infty}^{\infty} \frac{e^{-i n c \beta} d\beta}{(i\beta)^s} = \frac{-(n c)^s \pi}{|n c| (s-1)!} \quad \text{if } s \text{ is odd.}$$

It must be clearly understood that

$$\int_{-\infty}^{\infty} \frac{e^{-i n c \beta} d\beta}{(i\beta)^s}$$

is divergent but its contribution, so to speak, to the final result is that given.

Thus

$$\text{cont.} \int_{-\infty}^{\infty} e^{-i n \beta} \left( \bar{x} - \frac{1}{2} + \frac{r}{n} \right) \left( \frac{1}{i\beta} \right)^s d\beta = \frac{\left| n \left( \bar{x} - \frac{1}{2} + \frac{r}{n} \right) \right|^{s-1} \pi}{(s-1)!} \quad \text{if } s \text{ is even}$$

$$\text{and} \quad \frac{- \left\{ n \left( \bar{x} - \frac{1}{2} + \frac{r}{n} \right) \right\}^s \pi}{\left| n \left( \bar{x} - \frac{1}{2} + \frac{r}{n} \right) \right| (s-1)!} \quad \text{if } s \text{ is odd.}$$

We may write these formulae conveniently

$$\text{cont.} \int_{-\infty}^{\infty} e^{-i n \beta \left( \bar{x} - \frac{1}{2} + \frac{r}{n} \right)} \left( \frac{1}{i \beta} \right)^s d\beta = \pm \frac{n \left( \bar{x} - \frac{1}{2} + \frac{r}{n} \right)^{s-1} \pi}{(s-1)!} \text{ if } s \text{ is even}$$

$$\text{and } \mp \frac{n \left( \bar{x} - \frac{1}{2} + \frac{r}{n} \right)^{s-1} \pi}{(s-1)!} \text{ if } s \text{ is odd}$$

where the upper sign is to be taken in front of the bracket if the expression within the bracket is positive and the lower sign in the contrary case.

Thus we see that the distribution of means is the sum of a finite number of terms of the type  $C \left( \bar{x} - \frac{1}{2} + \frac{r}{n} \right)^s$  and that there will be points where the higher differential coefficients become discontinuous at

$$\bar{x} = 0, \pm \frac{1}{n}, \pm \frac{2}{n}, \dots \pm \frac{n-2}{2n} \text{ when } n \text{ is even}$$

$$\text{and } \bar{x} = \pm \frac{1}{2n}, \pm \frac{3}{2n}, \pm \frac{5}{2n}, \dots \pm \frac{n-2}{2n} \text{ when } n \text{ is odd, the}$$

origin being at the centre of the range.

The distribution thus consists of separate arcs of parabolae of degree  $n$  ( $p + q - 1$ ) — 1 having contact of order not less than  $(nt - 2)$  at the above points where they join,  $t$  being the smaller of  $p$  and  $q$ . These are the same points at which the distribution of means of samples from Type II and from rectangular distributions have discontinuous differential coefficients.

This might have been anticipated from HALL'S (16) geometrical treatment of the rectangular case. In determining the distribution of means of all the limited range curves we have to integrate a "density function" over a region defined by the intersection of a hyperplane with a hyper-cube and these discontinuities occur when the "polygon" of intersection changes Type. The values of the mean where this happens are quite independent of the particular "density function" integrated, that is of the form of the original frequency distribution.

(B) *Particular Cases.*

Particular cases of the general solution will now be considered.

(i)  $p = 1, q = 1$

This is the case of the rectangular population and has already been discussed by HALL (16) and the present writer (14).

(ii)  $p = 1, q = 2$

The original distribution is a triangle with a finite ordinate at one end of the range whose equation is given by

$$y = 2(1 - x)$$

We have

$$\frac{M_{-\frac{1}{2}, 1}(-\alpha)}{(-\alpha)^{1/2}} = -2e^{-\frac{1}{2}\alpha} \left( \frac{1}{\alpha} + \frac{1}{\alpha^2} \right) + 2 \frac{e^{\frac{1}{2}\alpha}}{\alpha^2}$$

So that the distribution of means is given by

$$y = \frac{n 2^{n-1}}{\pi} \int_{-\infty}^{\infty} \frac{e^{-i n \beta \bar{x}}}{(i \beta)^{2n}} \left[ e^{\frac{1}{2} i \beta} - e^{-\frac{1}{2} i \beta} (1 + i \beta) \right]^n d\beta$$

and the expression in squared brackets

$$\begin{aligned} &= \sum_{r=0}^n (-1)^r {}^nC_r \left( e^{\frac{1}{2} i \beta} \right)^{n-2r} (1 + i \beta)^r \\ &= \sum_{r=0}^n (-1)^r {}^nC_r \sum_{s=0}^r {}^rC_s \left( e^{\frac{1}{2} i \beta} \right)^{n-2r} (i \beta)^{2s} \end{aligned}$$

and the distribution of means is given by

$$y = \frac{n 2^{n-1}}{\pi} \sum_{r=0}^n (-1)^r {}^nC_r \sum_{s=0}^r {}^rC_s \left\{ \text{cont.} \int_{-\infty}^{\infty} \frac{e^{-i n \beta \left( \bar{x} - \frac{1}{2} + \frac{r}{n} \right)}}{(i \beta)^{2n-s}} d\beta \right\}$$

or

$$y = n 2^{n-1} \sum_{r=0}^n (-1)^r {}^nC_r \sum_{s=0}^r (-1)^{2n-s} {}^rC_s \left\{ \pm \frac{(n \bar{x} - \frac{n}{2} + r)^{2n-s-1}}{(2n-s-1)!} \right\}$$

where the upper sign is to be taken if the expression within the bracket is positive and the lower sign in the contrary case.

Putting  $n = 2, 3, 4$  we obtain the distribution of means for samples of 2, 3 and 4 respectively. These are

*Samples of 2.*

$$y = 4 \left[ -\frac{(2\bar{x}-1)^3}{3!} - 2 \left\{ \pm \frac{(2\bar{x})^3}{3!} \mp \frac{(2\bar{x})^2}{2!} \right\} + \right. \\ \left. + \frac{(2\bar{x}+1)^3}{3!} - \frac{2(2\bar{x}+1)^2}{2!} + \frac{(2\bar{x}+1)}{1!} \right] \dots\dots (14)$$

This is a curve with zero ordinate at both ends of the range and the two cubics which form it have contact of the first order at  $x = 0$ .

*Samples of 3.*

$$y = 12 \left[ -\frac{\left(3\bar{x}-\frac{3}{2}\right)^5}{5!} - 3 \left\{ \pm \frac{\left(3\bar{x}-\frac{1}{2}\right)^5}{5!} \mp \frac{\left(3\bar{x}-\frac{1}{2}\right)^4}{4!} \right\} \right. \\ \left. + 3 \left\{ \pm \frac{\left(3\bar{x}+\frac{1}{2}\right)^5}{5!} \mp \frac{2\left(3\bar{x}+\frac{1}{2}\right)^4}{4!} \pm \frac{\left(3\bar{x}+\frac{1}{2}\right)^3}{3!} \right\} \right. \\ \left. - \left\{ \frac{\left(3\bar{x}+\frac{3}{2}\right)^5}{5!} - \frac{3\left(3\bar{x}+\frac{3}{2}\right)^4}{4!} + \frac{3\left(3\bar{x}+\frac{3}{2}\right)^3}{3!} - \frac{\left(3\bar{x}+\frac{3}{2}\right)^2}{2!} \right\} \right] \\ \dots\dots (15)$$

This again has zero ordinate at both ends of the range, the points of junction of the separate arcs are at  $\bar{x} = \pm \frac{1}{6}$  but while there is 4 point contact at  $\bar{x} = \frac{1}{6}$ , there is only 3 point contact at  $\bar{x} = -\frac{1}{6}$ .

Samples of 4.

$$\begin{aligned}
 y = 32 \left[ -\frac{(4\bar{x}-2)^7}{7!} - 4 \left\{ \pm \frac{(4\bar{x}-1)^7}{7!} \mp \frac{(4\bar{x}-1)^6}{6!} \right\} \right. \\
 \left. + 6 \left\{ \pm \frac{(4\bar{x})^7}{7!} \mp \frac{2(4\bar{x})^6}{6!} \pm \frac{(4\bar{x})^5}{5!} \right\} \right. \\
 \left. - 4 \left\{ \pm \frac{(4\bar{x}+1)^7}{7!} \mp \frac{3(4\bar{x}+1)^6}{6!} \pm \frac{3(4\bar{x}+1)^5}{5!} \mp \frac{(4\bar{x}+1)^4}{4!} \right\} \right. \\
 \left. + \frac{(4\bar{x}+2)^7}{7!} - \frac{4(4\bar{x}+2)^6}{6!} + \frac{6(4\bar{x}+2)^5}{5!} - \frac{4(4\bar{x}+2)^4}{4!} + \frac{(4\bar{x}+2)^3}{3!} \right] \\
 \dots (16)
 \end{aligned}$$

This also has a zero ordinate at both ends of the range, the points of junction of the separate arcs are at  $\bar{x} = 0$  and  $\bar{x} = \pm \frac{1}{4}$  with 5, 6 and 4 point contact respectively at these points.

In all the cases which follow the distributions of means have a zero ordinate at both ends of the range and (except for samples of 2 when  $p$  or  $q = 1$ ) the axis of  $x$  is tangential to them, while the order of the contact at the junctions may be seen at once by inspection. Further where there is an ambiguous sign, the correct sign is determined by the rule that the upper sign is to be taken where the expression within the bracket following is positive and the lower sign in the contrary case. To save writing the expressions out at length they will now be expressed in the form

$$y = k \sum \lambda_{a,s} \left( \frac{n\bar{x} + a}{s!} \right)^s$$

accompanied by a table giving the coefficients  $\lambda_{a,s}$  for such values of  $a$  and  $s$  as occur. Where there is an ambiguous sign the upper sign must be taken when  $n\bar{x} + a$  is positive and the lower when  $n\bar{x} + a$  is negative.

(iii)  $p = 1, q = 3$ .

The population curve is a limited range  $J$  curve, with a finite or-

dinate at one end of the range, whose equation is  $y = 3 (1 - x)^2$ .  
We have

$$\frac{M_{-1, \frac{3}{2}}(-\alpha)}{(-\alpha)^2} = 3 \left[ \frac{2 e^{\frac{\alpha}{2}}}{\alpha^3} - \frac{e^{-\frac{1}{2}\alpha}}{\alpha} \left( 1 + \frac{2}{\alpha} + \frac{2}{\alpha^2} \right) \right]$$

$$\left[ \frac{M_{-1, \frac{3}{2}}(-\alpha)}{(-\alpha)^2} \right]^n = \left( \frac{3}{\alpha^3} \right)^n \left[ 2 e^{\frac{\alpha}{2}} - e^{-\frac{\alpha}{2}} (2 + 2\alpha + \alpha^2) \right]^n$$

and the distribution of means is given by

$$y = \frac{n}{2\pi} \int_{-\infty}^{\infty} e^{-n\beta \bar{x}} \frac{3^n}{(i\beta)^{3n}} \left[ 2 e^{\frac{1}{2}i\beta} - e^{-\frac{1}{2}i\beta} (2 + 2i\beta + (i\beta)^2) \right]^n d\beta$$

..... (17)

Putting  $n = 2, 3, 4$  in succession and in each case expanding and integrating we are led to the following results.

*Samples of 2*

$$y = 9 \sum \lambda_{a,s} \left( \frac{2\bar{x} + a}{s!} \right)^s$$

..... (18)

TABLE I. ...  $\lambda_{a,s}$  ( $p = 1, q = 3, n = 2$ ).

$a$	$s$				
	5	4	3	2	1
— 1	— 4	...	...	..	...
0	$\mp$ 8	$\pm$ 8	$\mp$ 4	...	...
1	+ 4	— 8	+ 8	— 4	+ 1

Where the ambiguous sign occurs, the upper sign is to be taken if  $(2\bar{x} + a)$  is positive and the lower sign if it is negative.

*Sample of 3.*

$$y = \frac{81}{2} \sum \lambda_{a,s} \frac{(3\bar{x} + a)^s}{s!}$$

..... (19)

TABLE II. ...  $\lambda_{a,s}$  ( $p = 1, q = 3, n = 3$ ).

$a$	$s$						
	8	7	6	5	4	3	2
$-\frac{3}{2}$	+ 8	...	...	...	...	...	...
$-\frac{1}{2}$	$\pm 24$	$\mp 24$	$\pm 12$	...	...	...	...
$+\frac{1}{2}$	$\mp 24$	$\pm 48$	$\mp 48$	$\pm 24$	$\mp 6$	...	...
$+\frac{3}{2}$	+ 8	- 24	+ 36	- 232	+ 18	- 6	+ 1

Where the ambiguous sign occurs, the upper sign is to be taken if  $(3\bar{x} + a)$  is positive and the lower sign if it is negative.

Sample of 4.

$$y = 162 \sum \lambda_{a,s} \frac{(4\bar{x} + a)^s}{s!} \dots (20)$$

TABLE III. ...  $\lambda_{a,s}$  ( $p = 1, q = 3, n = 4$ ).

$a$	$s$								
	11	10	9	8	7	6	5	4	3
- 2	- 16	...	...	...	..	...	...	...	...
- 1	$\mp 64$	$\pm 64$	$\mp 32$	...	...	...	...	..	...
0	$\pm 96$	$\mp 192$	$\pm 192$	$\mp 96$	$\pm 24$	...	...	...	...
1	$\mp 64$	$\pm 192$	$\mp 288$	$\pm 256$	$\mp 144$	$\pm 48$	$\mp 8$	...	...
2	+ 16	- 64	+ 128	- 160	+ 136	- 80	+ 32	- 8	+ 1

Where the ambiguous sign occurs the upper sign is to be taken if  $(4x + a)$  is positive and the lower sign if it is negative.

(iv)  $p = 1, q = 4$

The population curve is a limited range  $J$  curve with a finite ordinate at one end given by

$$y = 4(1 - x)^3$$

We have

$$\left[ \frac{M_{-\frac{3}{2}, 2}(-\alpha)}{(-\alpha)^{\frac{5}{2}}} \right] = 4 \left[ \frac{6e^{\frac{1}{2}\alpha}}{\alpha^4} - e^{-\frac{1}{2}\alpha} \left( \frac{1}{\alpha} + \frac{3}{\alpha^2} + \frac{6}{\alpha^3} + \frac{6}{\alpha^4} \right) \right]$$

$$\left[ \frac{M_{-\frac{3}{2}, 2}(-\alpha)}{(-\alpha)^{\frac{5}{2}}} \right]^n = \frac{4^n}{\alpha^{4n}} \left[ 6e^{\frac{1}{2}\alpha} - e^{-\frac{1}{2}\alpha} (6 + 6\alpha + 3\alpha^2 + \alpha^3) \right]^n$$

and the distribution of means is given by

$$y = \frac{n}{2\pi} \int_{-\infty}^{\infty} e^{-i\pi\beta x} \frac{4^n}{(i\beta)^{4n}} \left[ 6e^{\frac{1}{2}\beta} - e^{-\frac{1}{2}\beta} (6 + 6i\beta + 3(i\beta)^2 + (i\beta)^3) \right]^n d\beta$$

..... (21)

Putting  $n = 2, 3, 4$  in succession and expanding we obtain:—

*Samples of 2.*

$$y = 16 \sum \lambda_{a,s} \frac{(2\bar{x} + a)^s}{s!}$$

TABLE IV. ...  $\lambda_{a,s}$  ( $p = 1, q = 4, n = 2$ ).

a	s						
	7	6	5	4	3	2	1
— 1	— 36	...	...	...	...	...	...
0	± 72	± 72	± 36	± 12	...	...	...
1	+ 36	— 72	+ 72	— 48	+ 21	— 6	+ 1

..... (22)

Where the ambiguous sign occurs the upper sign is to be taken if  $(2\bar{x} + a)$  is positive and the lower sign if it is negative.

*Samples of 3.*

$$y = 96 \sum \lambda_{a,s} \frac{(3\bar{x} + a)^s}{s!}$$



TABLE V. ...  $\lambda_{a,s}$  ( $p = 1, q = 4, n = 3$ ).

$a$	$s$									
	11	10	9	8	7	6	5	4	3	2
$-\frac{3}{2}$	- 216	...	...	...	...	...	...	...	...	...
$-\frac{1}{2}$	$\mp 648$	$\pm 648$	$\mp 324$	$\pm 108$	...	...	...	...	...	...
$+\frac{1}{2}$	$\pm 648$	$\mp 1,296$	$\pm 1,296$	$\mp 864$	$\pm 378$	$\mp 108$	$\pm 18$	...	...	...
$+\frac{3}{2}$	- 216	+ 648	- 972	+ 972	- 702	+ 378	- 153	+ 45	- 9	+ 1

..... (23)

Where the ambiguous sign occurs the upper sign is to be taken if  $(3\bar{x} + a)$  is positive and the lower sign if it is negative.

Samples of 4.

$$y = 512 \sum \lambda_{a,s} \frac{(4\bar{x} + a)^s}{s!}$$

TABLE VI. ...  $\lambda_{a,s}$  ( $p = 1, q = 4, n = 4$ ).

$a$	$s$					
	15	14	13	12	11	10
- 2	- 1,296	...	...	...	...	...
- 1	$\mp 5,184$	$\pm 5,184$	$\mp 2,592$	$\pm 864$	...	...
0	$\pm 7,776$	$\mp 15,552$	$\pm 15,552$	$\mp 10,368$	$\pm 4,536$	$\mp 1,296$
1	$\mp 5,184$	$\pm 15,552$	$\mp 23,328$	$\pm 23,328$	$\mp 16,848$	$\pm 9,072$
2	+ 1,296	- 5,184	+ 10,368	- 13,824	+ 13,608	- 10,368

$s$						
9	8	7	6	5	4	3
...	...	...	...	...	...	...
...	...	...	...	...	...	...
$\pm 216$	...	...	...	...	...	...
$\mp 3,672$	$\pm 1,080$	$\mp 216$	$\pm 24$	...	...	...
+ 6,264	- 3,024	+ 1,161	- 348	+ 78	- 12	+ 1

..... (24)

Where the ambiguous sign occurs the upper sign is to be taken if  $(4\bar{x} + a)$  is positive, and the lower sign if it is negative.

(v)  $p = 2, q = 2$

Here the population curve is the Type II curve  $y = 6x(1 - x)$  and this case is of sufficient interest to be considered in some detail. We have

$$\begin{aligned} \left[ \frac{M_{0,2}(-\alpha)}{(-\alpha)^2} \right] &= 6 \left\{ \frac{e^{-\frac{1}{2}\alpha}}{\alpha^2} \left( 1 + \frac{2}{\alpha} \right) + \frac{e^{\frac{1}{2}\alpha}}{\alpha^2} \left( 1 - \frac{2}{\alpha} \right) \right\} \\ &= \frac{6}{\alpha^3} \left\{ e^{-\frac{1}{2}\alpha} (2 + \alpha) + e^{\frac{1}{2}\alpha} (-2 + \alpha) \right\} \end{aligned}$$

Whence the distribution of means may be written

$$y = \frac{n}{2\pi} \int_{-\infty}^{\infty} e^{-in\beta\bar{x}} \left( \frac{6}{i\beta^3} \right)^n \left\{ e^{-\frac{1}{2}i\beta} (2 + i\beta) + e^{\frac{1}{2}i\beta} (-2 + i\beta) \right\}^n d\beta (*)$$

..... (25)

On expanding the Binomial this may be written

$$y = \frac{n 6^n}{2\pi} \sum_{r=0}^n (-1)^r {}^nC_r f_{n,r}(\bar{x})$$

where

$$f_{n,r}(\bar{x}) \left\{ \text{cont.} \int_{-\infty}^{\infty} \frac{e^{-in\beta\bar{x} + \frac{1}{2}in-r}}{(i\beta)^3} \sum_{s=0}^n (-1)^s 2^{n-s} p_{r,s}(i\beta)^s d\beta \right\}.$$

(\*) This may also be written

$$\begin{aligned} y &= \frac{n}{2\pi} \int_{-\infty}^{\infty} e^{-in\beta\bar{x}} \left( \frac{6}{i\beta^3} \right)^n \left\{ -4 \sinh \frac{i\beta}{2} + 2i\beta \cosh \frac{i\beta}{2} \right\}^n d\beta \\ &= \frac{n}{2\pi} \cdot 12^n \int_{-\infty}^{\infty} e^{-in\beta\bar{x}} \left( \frac{2 \sin \frac{\beta}{2} - \beta \cos \frac{\beta}{2}}{\beta^3} \right)^n d\beta \end{aligned}$$

a form which is easily deduced from the BESSEL formula for the Type II case previously established.

and

$$p_{r,s} = {}^{n-r}C_0 {}^rC_s - {}^{n-r}C_1 {}^rC_{s-1} + \\ + \dots + (-1)^r {}^{n-r}C_r {}^rC_{s-r} \dots + (-1)^s {}^{n-r}C_s {}^rC_0$$

with the convention that  ${}^nC_b = 0$  if  $b > a$  or

$$y = \frac{n 6^n}{2} \sum_{r=0}^n (-1)^r {}^nC_r \sum_{s=0}^n (-1)^s 2^{n-s} p_{r,s} \left\{ \pm \frac{\left( n\bar{x} + \frac{1}{2} n - r \right)^{3n-s-1}}{(3n-s-1)!} \right\}$$

or since

$${}^nC_r p_{r,s} = (-1)^{r+s} {}^nC_s p_{s,r} (*) \\ y = \frac{n 6^n}{2^2 n} \sum_{s=0}^n (-1)^{n-s} \frac{{}^nC_s}{(3n-s-1)!} \\ \sum_{r=0}^n p_{s,r} \left\{ \pm (2n\bar{x} + n - 2r)^{3n-s-1} \right\} \dots (26)$$

a general solution for this case.

(\*) Suppose  $s > r$  then

$$\begin{aligned} {}^nC_r p_{r,s} &= \\ &= {}^nC_r \sum_{t=0}^{s-r} (-1)^t {}^{n-r}C_t {}^rC_{s-t} \\ &= {}^nC_r \sum_{t=s-r}^{s-r} (-1)^t {}^{n-r}C_t {}^rC_{s-t} \\ &= (-1)^{s-r} {}^{n-r}C_{s-r} {}^nC_r \sum_{t=s-r}^{s-r} (-1)^{t-s+r} \frac{(n-s)(n-s-1)\dots(n-r-t+1)}{(s-r+1)(s-r+2)\dots t} {}^rC_{s-t} \\ &= (-1)^{s-r} {}^nC_s \sum_{t=s-r}^{s-r} {}^sC_r (-1)^{t-s+r} \frac{(n-s)(n-s-1)\dots(n-r-t+1)}{(s-r+1)(s-r+2)\dots t} {}^rC_{s-t} \\ &= (-1)^{s-r} {}^nC_s \sum_{t=s-r}^{s-r} (-1)^{t-s+r} \frac{s!}{t!(s-t)!} {}^{n-s}C_{r+t-s} \\ &= (-1)^{s-r} {}^nC_s p_{s,r} \\ &= (-1)^{s+r} {}^nC_s p_{s,r} \end{aligned}$$

Similarly the result follows if  $r > s$  and is obvious if  $r = s$ .

There is an alternative form in which this may be put.

Since  $p_{s,r} = (-1)^{n-s} p_{s,n-r}$

$$\begin{aligned} & [p_{s,r} \{ \pm (2n\bar{x} + n - 2r)^{3^{n-s-1}} \} + \\ & p_{s,n-r} \{ \pm (2n\bar{x} - n + 2r)^{3^{n-s-1}} \}] \\ & = p_{s,r} [ \{ \pm (2n\bar{x} + n - 2r)^{3^{n-s-1}} \} + \\ & \{ \pm (-2n\bar{x} + n - 2r)^{3^{n-s-1}} \} ] \end{aligned}$$

Whence we may write

$$y = \frac{n \cdot 6^n}{2^{2n}} \sum_{s=0}^n \frac{(-1)^{n-s} C_s}{(3n-s-1)!} \sum_{r=0}^{\frac{n-1}{2}} p_{s,r} f(r, \bar{x})$$

where

$$f(r, \bar{x}) = \begin{cases} \pm (2n\bar{x} + n - 2r)^{3^{n-s-1}} \\ \pm (-2n\bar{x} + n - 2r)^{3^{n-s-1}} \end{cases} \quad \text{if } n \text{ is odd}$$

and

$$y = \frac{n \cdot 6^n}{2^{2n}} \sum_{s=0}^n \frac{(-1)^{n-s} C_s}{(3n-s-1)!} \left\{ \begin{aligned} & \pm p_{s, \frac{n}{2}} (2n\bar{x})^{3^{n-s-1}} \\ & + \sum_{r=0}^{\frac{n}{2}-1} p_{s,r} f(r, \bar{x}) \end{aligned} \right\} \quad \text{if } n \text{ is even} \quad (26 \text{ bis})$$

a form first deduced from the trigonometrical expression for the Type II case previously established, and somewhat analogous to the solution obtained for the rectangle. In practice it is easier to substitute directly for  $n$  in (25) above, expand, and then integrate rather than to use the general solution which of course leads to the same results. On putting  $n = 2, 3, 4$  in succession we find :—

Samples of 2:

$$y = 36 \sum \lambda_{a,s} \frac{(2\bar{x} + a)^s}{s!}$$

TABLE VII. ...  $\lambda_{a,s}$  ( $p = 2, q = 2, n = 2$ ).

a	s		
	5	4	3
— 1	— 4	— 4	— 1
0	± 8	...	± 2
1	+ 4	— 4	+ 1

..... (27)

Where the ambiguous sign occurs the upper sign is to be taken if  $(2\bar{x} + a)$  is positive and the lower sign if it is negative.

Samples of 3.

$$y = 324 \sum \lambda_{a,s} \frac{(3\bar{x} + a)^s}{s!}$$

TABLE VIII. ...  $\lambda_{a,s}$  ( $p = 2, q = 2, n = 3$ ).

$a$	$s$			
	8	7	6	5
$-\frac{3}{2}$	— 8	— 12	— 6	— 1
$-\frac{1}{2}$	$\mp$ 24	$\mp$ 12	$\pm$ 6	$\pm$ 3
$\frac{1}{2}$	$\pm$ 24	$\mp$ 12	$\mp$ 6	$\pm$ 3
$\frac{3}{2}$	— 8	+ 12	— 6	+ 1

..... (28)

Where the ambiguous sign occurs the upper sign is to be taken if  $(3\bar{x} + a)$  is positive and the lower sign if it is negative.

Samples of 4.

$$y = 2592 \sum \lambda_{a,s} \left( \frac{4\bar{x} + a}{s!} \right)^s$$

TABLE IX. ...  $\lambda_{a,s}$  ( $p = 2, q = 2, n = 4$ ).

$a$	$s$				
	11	10	9	8	7
— 2	— 16	— 32	— 24	— 8	— 1
— 1	$\mp$ 64	$\mp$ 64	—	$\pm$ 16	$\pm$ 4
0	$\pm$ 96	...	$\mp$ 48	...	$\pm$ 6
1	$\mp$ 64	$\pm$ 64	...	$\mp$ 16	$\pm$ 4
2	+ 16	— 32	+ 24	— 8	+ 1

..... (29)

Where the ambiguous sign occurs the upper sign is to be taken if  $(4\bar{x} + a)$  is positive and the lower sign if it is negative.

(vi)  $p = 2, q = 3$

The population curve is

$$y = 12x(1-x)^2$$

We have

$$\begin{aligned} \left[ \frac{M_{-\frac{1}{2}, 2}^{(-\alpha)}}{(-\alpha)^{\frac{5}{2}}} \right] &= 12e^{-\frac{1}{2}\alpha} \left( \frac{1}{\alpha^2} + \frac{4}{\alpha^3} + \frac{6}{\alpha^4} \right) + 24e^{\frac{\alpha}{2}} \left( \frac{1}{\alpha^3} - \frac{3}{\alpha^4} \right) \\ &= \frac{12}{\alpha^4} \left[ e^{-\frac{1}{2}\alpha} (6 + 4\alpha + \alpha^2) - 2e^{\frac{1}{2}\alpha} (3 - \alpha) \right] \end{aligned}$$

and the distribution of means is

$$\begin{aligned} y = \frac{n}{2\pi} \int_{-\infty}^{\infty} e^{-i\pi\beta\bar{x}} \left[ \frac{12}{(i\beta)^4} \left\{ e^{-\frac{1}{2}i\beta} (6 + 4i\beta + (i\beta)^2) - \right. \right. \\ \left. \left. - 2e^{\frac{1}{2}i\beta} (3 - i\beta) \right\} \right]^n d'\beta \end{aligned}$$

On putting  $n = 2, 3, 4$  in succession we obtain

*Samples of 2.*

$$y = 12^2 \sum \lambda_{a,s} \frac{(2\bar{x} + a)^s}{s!}$$

TABLE X. ...  $\lambda_{a,s}$  ( $p = 2, q = 3, n = 2$ ).

a	s				
	7	6	5	4	3
— 1	— 36	— 24	— 4	...	...
0	± 72	± 24	± 4	± 4	...
1	+ 36	— 48	+ 28	— 8	+ 1

..... (30)

Where the ambiguous sign occurs the upper sign is to be taken if  $(2\bar{x} + a)$  is positive and the lower sign if it is negative.

Samples of 3.

$$y = \frac{3 \cdot 12^3}{2} \sum \lambda_{a,s} \frac{(3\bar{x} + a)^s}{s!}$$

TABLE XI. ...  $\lambda_{a,s}$  ( $p = 2, q = 3, n = 3$ ).

a	s						
	11	10	9	8	7	6	5
$-\frac{3}{2}$	+ 216	+ 216	+ 72	+ 8	...	...	...
$-\frac{1}{2}$	± 648	...	∓ 108	± 24	± 12	...	...
$\frac{1}{2}$	∓ 648	± 648	∓ 216	∓ 24	± 30	∓ 6	...
$\frac{3}{2}$	+ 216	− 432	+ 396	− 208	+ 66	− 12	+ 1

..... (31)

Where the ambiguous sign occurs the upper sign is to be taken if  $(3\bar{x} + a)$  is positive and the lower sign if it is negative.

Samples of 4.

$$y = 2 (12)^4 \sum \lambda_{a,s} \frac{(4\bar{x} + a)^s}{s!}$$

TABLE XII. ...  $\lambda_{a,s}$  ( $p = 2, q = 3, n = 4$ ).

a	s								
	15	14	13	12	11	10	9	8	7
− 2	− 1,296	− 1,728	− 864	− 192	− 16	...	...	...	...
− 1	∓ 5,184	∓ 1,728	± 864	± 96	∓ 160	∓ 32	...	...	...
0	± 7,776	∓ 5,184	...	± 1,152	∓ 264	∓ 48	± 24	...	...
1	∓ 5,184	± 8,640	∓ 6,048	± 1,824	± 80	∓ 240	± 72	∓ 8	...
2	+ 1,296	− 3,456	+ 4,320	− 3,264	+ 1,624	− 544	+ 120	− 16	+ 1

..... (32)

Where the ambiguous sign occurs the upper sign is to be taken if  $(4\bar{x} + a)$  is positive and the lower sign if it is negative.

(vii)  $p = 2, q = 4$ .

The population curve is

$$y = 20 x (1 - x)^3$$

We have

$$\left[ \frac{M_{-1, \frac{5}{2}}(-\alpha)}{(-\alpha)^3} \right] = \frac{20}{\alpha^3} \left\{ -6 e^{\frac{1}{2}\alpha} (4 - \alpha) + e^{-\frac{1}{2}\alpha} (24 + 18\alpha + 6\alpha^2 + \alpha^3) \right\}$$

whence the distribution of means is given by

$$y = \frac{n}{2\pi} \int_{-\infty}^{\infty} e^{-i n \beta \bar{x}} \left[ \frac{20}{(i\beta)^3} \left\{ -6 e^{\frac{1}{2}i\beta} (4 - i\beta) + e^{-\frac{1}{2}i\beta} (24 + 18(i\beta) + 6(i\beta)^2 + (i\beta)^3) \right\} \right]^n d\beta \quad \dots (33)$$

On putting  $n = 2, 3, 4$  in succession we obtain :—

*Samples of 2.*

$$y = 20^2 \sum \lambda_{s,s} \frac{(2\bar{x} + a)^s}{s!}$$

TABLE XIII. ...  $\lambda_{s,s}$  ( $p = 2, q = 4, n = 2$ ).

s	s						
	9	8	7	6	5	4	3
— 1	— 576	— 288	— 36	...	...	...	...
0	± 1,152	± 576	± 72	± 24	± 12	...	...
1	+ 576	— 864	+ 612	— 264	+ 72	— 12	+ 1

..... (34)

Where the ambiguous sign occurs the upper sign is to be taken if  $(2\bar{x} + a)$  is positive and the lower sign if it is negative.



Samples of 3.

$$y = \frac{3}{2} (20)^3 \sum \lambda_{a,s} \frac{(3\bar{x} + a)^s}{s!}$$

TABLE XIV. ...  $\lambda_{a,s}$  ( $p = 2, q = 4, n = 3$ ).

a	s									
	14	13	12	11	10	9	8	7	6	5
$-\frac{3}{2}$	-13,824	-10,368	-2,592	-216	...	...	...	...	...	...
$-\frac{1}{2}$	$\mp 41,472$	$\pm 10,368$	$\pm 2,592$	$\mp 1,512$	$\pm 216$	$\pm 108$	...	..	...	...
$\frac{1}{2}$	$\pm 41,472$	$\mp 51,840$	$\pm 28,512$	$\mp 7,992$	$\pm 432$	$\pm 432$	$\mp 144$	$\pm 18$	...	...
$\frac{3}{2}$	-13,824	+31,104	-33,696	+23,112	-11,016	+3,780	-936	+162	-18	+1

..... (35)

Where the ambiguous sign occurs the upper sign is to be taken if  $(3\bar{x} + a)$  is positive and the lower sign if it is negative.

Samples of 4.

$$y = 2 (20)^4 \sum \lambda_{a,s} \frac{(4\bar{x} + a)^s}{s!}$$

TABLE XV ...  $\lambda_{a,s}$  ( $p = 2, q = 4, n = 4$ ).

a	s				
	19	18	17	16	15
-2	-331,776	-331,776	-124,416	-20,736	-1,296
-1	$\mp 1,327,104$	...	$\pm 165,888$	$\mp 27,648$	$\mp 5,184$
0	$\pm 1,990,656$	$\mp 1,990,656$	$\pm 746,496$	$\mp 41,472$	$\mp 75,168$
1	$\mp 1,327,104$	$\pm 2,654,208$	$\mp 2,468,320$	$\pm 1,410,048$	$\mp 502,848$
2	+331,776	-995,328	+1,451,520	-1,361,664	+913,680

s							
14	13	12	11	10	9	8	7
...	...	...	...	...	...	...	...
$\pm 5,184$	$\pm 864$	...	...	...	...	...	...
$\pm 25,920$	$\mp 1,728$	$\mp 864$	$\pm 216$	...	...	...	...
$\pm 98,496$	$\pm 864$	$\mp 6,912$	$\pm 2,160$	$\mp 336$	$\pm 24$	...	...
-461,376	+179,712	-54,432	+12,744	-2,256	+288	-24	+1

..... (36)

Where the ambiguous sign occurs the upper sign is to be taken if  $(4 \bar{x} + a)$  is positive and the lower sign if it is negative.

(viii)  $p = 3, q = 3$ .

This is the case of the symmetrical Type II curve

$$y = 30 x^2 (1 - x)^2$$

We have

$$\left[ \frac{M_{0, \frac{1}{2}}(-\alpha)}{(-\alpha)^3} \right] = \frac{60}{\alpha^5} \left[ e^{\frac{\alpha}{2}} (12 - 6\alpha + \alpha^2) - e^{-\frac{\alpha}{2}} (12 + 6\alpha + \alpha^2) \right]$$

and the distribution of means is given by

$$y = \frac{n}{2\pi} \int_{-\infty}^{\infty} e^{-in\beta x} \left[ \frac{60}{(i\beta)^5} \left\{ e^{i\beta} (12 - 6i\beta + (i\beta)^2) - e^{-i\beta} (12 + 6i\beta + (i\beta)^2) \right\} \right]^n d\beta \dots\dots 37$$

On putting  $n = 2, 3, 4$  in succession we obtain :—

*Samples of 2.*

$$y = 60^2 \sum \lambda_{a,s} \frac{(2\bar{x} + a)^s}{s!}$$

TABLE XVI. ...  $\lambda_{a,s}$  ( $p = 3, q = 3, n = 2$ ).

a	s				
	9	8	7	6	5
— 1	— 144	— 144	— 60	— 12	— 1
o	± 288	...	± 24	...	± 2
1	+ 144	— 144	+ 60	— 12	+ 1

..... (38)

Where the ambiguous sign occurs the upper sign is to be taken if  $(2 \bar{x} + a)$  is positive and the lower sign if it is negative.

Samples of 3.

$$y = \frac{3}{2} (60)^3 \sum \lambda_{a,s} \frac{(3\bar{x} + a)^s}{s!}$$

TABLE XVII. ...  $\lambda_{a,s}$  ( $p = 3, q = 3, n = 3$ ).

$a$	$s$						
	14	13	12	11	10	9	8
$-\frac{3}{2}$	+ 1,728	+ 2,592	+ 1,728	+ 648	+ 144	+ 18	+ 1
$-\frac{1}{2}$	$\pm$ 5,184	$\pm$ 2,592	...	$\mp$ 216	...	$\pm$ 18	$\pm$ 3
$+\frac{1}{2}$	$\mp$ 5,184	$\pm$ 2,592	...	$\mp$ 216	...	$\pm$ 18	$\mp$ 3
$+\frac{3}{2}$	+ 1,728	- 2,592	+ 1,728	- 648	+ 144	- 18	+ 1

..... (39)

Where the ambiguous sign occurs the upper sign is to be taken if  $(3\bar{x} + a)$  is positive and the lower sign if it is negative.

Samples of 4.

$$y = 2 (60)^4 \sum \lambda_{a,s} \frac{(4\bar{x} + a)^s}{s!}$$

TABLE XVIII ...  $\lambda_{a,s}$  ( $p = 3, q = 3, n = 4$ )

$a$	$s$								
	19	18	17	16	15	14	13	12	11
-2	- 20,736	- 41,472	- 38,016	- 20,736	- 7,344	- 1,728	- 264	- 24	- 1
-1	$\mp$ 82,944	$\mp$ 82,944	$\mp$ 27,648	...	$\pm$ 1,728	...	$\mp$ 192	$\mp$ 48	$\mp$ 4
0	$\pm$ 124,416	...	$\mp$ 20,736	...	$\pm$ 2,592	...	$\mp$ 144	...	$\pm$ 6
1	$\mp$ 82,944	$\pm$ 82,944	$\mp$ 27,648	...	$\pm$ 1,728	...	$\mp$ 192	$\pm$ 48	$\mp$ 4
2	+ 20,736	- 41,472	+ 38,016	- 20,736	+ 7,344	- 1,728	+ 264	- 24	+ 1

..... (40)

Where the ambiguous sign occurs the upper sign is to be taken if  $(4\bar{x} + a)$  is positive and the lower sign if it is negative.

(ix)  $p = 3, q = 4$ .

The population curve is given by  $y = 15x^2 (1-x)^3$ . We have

$$\left[ \frac{M_{-\frac{1}{2}, 3}(-\alpha)}{(-\alpha)_2^7} \right] = \frac{120}{\alpha^6} \left[ 3 e^{\frac{1}{2}\alpha} (20 - 8\alpha + \alpha^2) - e^{-\frac{1}{2}\alpha} (60 + 36\alpha + 9\alpha^2 + \alpha^3) \right]$$

and the distribution of means is given by

$$y = \frac{n}{2\pi} \int_{-\infty}^{\infty} e^{-in\beta x} \left[ \frac{120}{(i\beta)^6} \left\{ 3 e^{\frac{1}{2}i\beta} (20 - 8\beta + (i\beta)^2) - e^{-\frac{1}{2}i\beta} (60 + 36(i\beta) + 9(i\beta)^2 + (i\beta)^3) \right\} \right]^n d\beta$$

..... (41)

Putting  $n = 2, 3, 4$  in succession we obtain the results tabulated in Tables XIX, XX and XXI.

*Samples of 2.*

$$y = (120)^2 \sum \lambda_{a,s} \frac{(2x+a)^s}{s!}$$

TABLE XIX. ...  $\lambda_{a,s}$  ( $p = 3, q = 4, n = 2$ )

a	s						
	11	10	9	8	7	6	5
- 1	- 3,600	- 2,880	- 936	- 144	- 9	...	...
0	± 7,200	± 1,440	± 288	± 96	± 6	± 6	...
1	+ 3,600	- 4,320	+ 2,376	- 768	+ 153	- 18	+ 1

..... (42)

Where the ambiguous sign occurs the upper sign is to be taken if  $(2\bar{x} + a)$  is positive and the lower sign if it is negative.

Samples of 3.

$$y = \frac{3}{2} (120)^3 \sum \lambda_{a,s} \frac{(3\bar{x} + a)^s}{s!}$$

TABLE XX. ...  $\lambda_{a,s}$  ( $p = 3, q = 4, n = 3$ ).

a	s			
	17	16	15	14
— $\frac{3}{2}$	— 216,000	— 259,200	— 136,080	— 39,744
— $\frac{1}{2}$	± 648,000	± 12,960	± 45,360	± 8,208
$\frac{1}{2}$	± 648,000	± 518,400	± 149,040	± 6,048
$\frac{3}{2}$	— 216,000	+ 388,800	— 330,480	+ 174,096

s					
13	12	11	10	9	8
— 6,804	— 648	— 27	...	...	...
± 2,700	± 108	± 189	± 27	...	...
± 6,372	± 864	± 261	± 90	± 9	...
— 62,532	+ 15,876	— 2,853	— 351	— 27	+ 1

..... (43)

Where the ambiguous sign occurs the upper sign is to be taken if  $(3\bar{x} + a)$  is positive and the lower sign if it is negative.

Samples of 4.

$$y = 2 (120)^4 \sum \lambda_{a,s} \frac{(4\bar{x} + a)^s}{s!}$$

TABLE XXI. ...  $\lambda_{a,s}$  ( $p = 3, q = 4, n = 4$ ).

a	s				
	23	22	21	20	19
— 2	— 12,960,000	— 20,736,000	— 15,033,600	— 6,428,160	— 1,770,336
— 1	± 51,840,000	± 31,104,000	± 3,110,400	± 1,589,760	± 228,096
0	± 77,760,000	± 31,104,000	± 3,110,400	± 3,317,760	± 160,704
1	± 51,840,000	± 72,576,000	± 44,582,400	± 14,722,560	± 2,260,224
2	+ 12,960,000	— 31,104,000	+ 35,769,600	— 26,058,240	+ 13,382,496

(continued)

s								
18	17	16	15	14	13	12	11	
— 321,408	— 37,584	— 2,592	— 81	...	...	...	...	
∓ 62,208	± 864	± 7,776	± 1,620	± 108	...	...	...	
∓ 238,464	± 34,560	± 6,912	∓ 1,674	∓ 108	± 54	...	...	
∓ 103,680	± 88,992	± 864	∓ 7,020	± 1,860	∓ 228	± 12	...	
— 5,101,056	+ 1,479,600	— 329,184	+ 55,809	— 7,044	+ 630	— 36	+ 1	..... (44)

Where the ambiguous sign occurs the upper sign is to be taken if  $(4\bar{x} + a)$  is positive and the lower sign if it is negative.

$(x) \, p = 4, q = 4.$

The population curve is here the symmetrical Type II curve

$$y = 140 \, x^3 (1 - x)^3$$

We have

$$\left[ \begin{matrix} M_0, \frac{7}{2} (-\alpha) \\ -(-\alpha)^4 \end{matrix} \right] = \frac{840}{\alpha^7} \left[ e^{-\frac{1}{2}} (120 + 60 \alpha + 12 \alpha^2 + \alpha^3) - e^{\frac{1}{2}} \alpha (120 - 60 \alpha + 12 \alpha^2 - \alpha^3) \right]$$

and the distribution of means is given by

$$y = \frac{n}{2\pi} \int_{-\infty}^{\infty} e^{-i n \beta \bar{x}} \left[ \frac{840}{(i\beta)^7} \left\{ e^{-\frac{1}{2} i \beta} (120 + 60 i \beta + 12 (i\beta)^2 + (i\beta)^3) - e^{\frac{1}{2} i \beta} (120 - 60 i \beta + 12 (i\beta)^2 - (i\beta)^3) \right\} \right]^n d\beta$$

..... (45)

Putting  $n = 2, 3, 4$  in succession we obtain the results tabulated in Tables XXII, XXIII, and XXIV.

Samples of 2

$$y = (840)^2 \sum \lambda_{a,s} \frac{(2\bar{x} + a)^s}{s!}$$

TABLE XXII. ...  $\lambda_{a,s}$  ( $p = 4, q = 4, n = 2$ ).

a	s						
	13	12	11	10	9	8	7
— 1	— 14,400	— 14,400	— 6,480	— 1,680	— 264	— 24	— 1
0	∓ 28,800	...	± 1,440	...	∓ 48	...	± 2
1	+ 14,400	— 14,400	+ 6,480	— 1,680	+ 264	— 24	+ 1

..... (46)

Where the ambiguous sign occurs the upper sign is to be taken if  $(2\bar{x} + a)$  is positive and the lower sign if it is negative.

Samples of 3.

$$y = \frac{3}{2} (840)^3 \Sigma \lambda_{a,s} \frac{(3\bar{x} + a)^s}{s!}$$

TABLE XXIII. ...  $\lambda_{a,s}$  ( $p = 4, q = 4, n = 3$ ).

a	s			
	20	19	18	17
— 3	— 1,728,000	— 2,592,000	— 1,814,400	— 777,600
2				
— 1	干 5,184,000	干 2,592,000	干 259,200	± 86,400
2				
1	± 5,184,000	干 2,592,000	± 259,200	± 86,400
2				
3	— 1,728,000	十 2,592,000	— 1,814,400	+ 777,600
2				

16	15	14	13	12	11
— 224,640	— 45,360	— 6,408	— 612	— 36	— 1
± 17,280	干 2,160	干 504	± 108	± 36	± 3
干 17,280	干 2,160	± 504	± 108	干 36	± 3
— 224,640	+ 45,360	— 6,408	+ 612	— 36	+ 1

..... (47)

Where the ambiguous sign occurs the upper sign is to be taken if  $(3\bar{x} + a)$  is positive and the lower sign if it is negative.

Samples of 4.

$$y = 2 (840)^4 \Sigma \lambda_{a,s} \frac{(4\bar{x} + a)^s}{s!}$$

TABLE XXIV ...  $\lambda_{a,s}$  ( $p = 4, q = 4, n = 4$ )

a	s				
	27	26	25	24	23
— 2	— 207,360,000	— 414,720,000	— 393,984,000	— 235,008,000	— 97,977,600
— 1	± 829,440,000	± 829,440,000	± 331,776,000	± 55,296,000	± 2,073,600
0	± 1,244,160,000	...	± 1,244,160,000	...	± 7,257,600
1	± 829,440,000	± 829,440,000	± 331,776,000	± 55,296,000	± 2,073,600
2	+ 207,360,000	— 414,720,000	+ 393,984,000	— 235,008,000	+ 97,977,600

22	21	20	19	18	17	16	15
— 30,067,200	— 6,963,840	— 1,226,880	— 163,296	— 16,032	— 1,104	— 48	— 1
± 2,073,600	± 138,240	± 34,560	± 3,456	± 4,416	± 960	± 96	± 4
...	± 380,160	...	± 12,096	...	± 288	...	± 6
∓ 2,073,600	± 138,240	± 34,560	± 3,456	± 4,416	± 960	∓ 96	± 4
— 30,067,200	+ 6,963,840	— 1,226,880	+ 163,296	— 16,032	+ 1,104	— 48	+ 1

..... (48)

Where the ambiguous sign occurs the upper sign is to be taken if  $(4\bar{x} + a)$  is positive and the lower sign if it is negative.

(C). *Moments of the Distribution of Means.*

The moments about its own mean of the distribution of means may be obtained in a variety of ways.

The simplest however seems to be to make use of the following relations (\*):

$$M_2 = \frac{\mu_2}{n}$$

$$M_3 = \frac{\mu_3}{n^2}$$

$$M_4 - 3 M_2^2 = \frac{\mu_4 - 3 \mu_2^2}{n^3}$$

$$M_5 - 10 M_3 M_2 = \frac{\mu_5 - 10 \mu_3 \mu_2}{n^4}$$

$$M_6 - 15 M_4 M_2 + 10 M_3^2 = \frac{\mu_6 - 15 \mu_4 \mu_2 + 10 \mu_3^2}{n^5}$$

(\*) See for instance (16), p. 243.

These results are most generally expressed by the relation

$$\kappa(1^r) = \frac{\kappa_r}{n^r - 1}$$

where  $\kappa(1^r)$  is the  $r^{\text{th}}$  semi-invariant of the distribution of means,  $\kappa_r$ , the  $r^{\text{th}}$  semi-invariant of the population.



$$M_7 - 21 M_5 M_2 - 35 M_4 M_3 + 210 M_3 M_2^2 = \frac{\mu_7 - 21 \mu_5 \mu_2 - 35 \mu_4 \mu_3 + 210 \mu_3 \mu_2^2}{n^6} \dots (49)$$

where  $M_s$  denotes the  $s^{\text{th}}$  moment about its own mean of the distribution of means,  $\mu_s$  of the population.

The first four moments of the distribution of means of Type I

$$y = \frac{\Gamma(p+q)}{\Gamma(p)\Gamma(q)} x^{p-1} (1-x)^{q-1}$$

will now be obtained :— For this case Pearson has given the results :—

$$\begin{aligned} \mu_2 &= \frac{\varepsilon b^2}{r^2 (r+1)} \\ \mu_3 &= \frac{2(q-p)\varepsilon \cdot b^3}{r^3 (r+1)(r+2)} \\ \mu_4 &= \frac{3\varepsilon(2r^2+r-6\varepsilon) \cdot b^4}{r^4 (r+1)(r+2)(r+3)} \end{aligned}$$

where  $\varepsilon = pq$ ,  $r = p+q$  and  $b$  is in this case unity, being the range of the curve.

Hence we obtain

$$\begin{aligned} M_2 &= \frac{1}{n} \frac{\varepsilon b^2}{r^2 (r+1)} \\ M_3 &= \frac{2}{n^2} \frac{(q-p)\varepsilon \cdot b^3}{r^3 (r+1)(r+2)} \\ M_4 - 3M_2^2 &= \frac{6\varepsilon}{n^3} \left\{ \frac{r^2(r+1) - \varepsilon(5r+6)}{r^4 (r+1)^2 (r+2)(r+3)} \right\} b^4 \end{aligned}$$

or

$$M_4 = \frac{6\varepsilon}{n^3} \left\{ \frac{r^2(r+1) - \varepsilon(5r+6)}{r^4 (r+1)^2 (r+2)(r+3)} + 3 \frac{\varepsilon^2 (r+2)(r+3)}{n \varepsilon^2 (r+2)(r+3)} \right\} b^4 \dots (51)$$

and

$$\left. \begin{aligned} B_1 &= \frac{4(q-p)^2(r+1)}{n\varepsilon(r+2)^2} \\ B_2 &= 3 + \frac{6\{r^2(r+1) - \varepsilon(5r+6)\}}{n\varepsilon(r+2)(r+3)} \end{aligned} \right\} \dots (52)$$

For the symmetrical Type II case  $p = q$ , the latter reduces to

$$B_1 = 0$$

$$B_2 = 3 - \frac{6}{n(2p+3)} \dots (52 \text{ bis})$$

and the approach to normality with increasing size of sample is rapid.

(D) *Summary to Part I.*

(i) The distribution of the means of random samples of  $n$  from a population, supposed indefinitely large of the Type

$$y = \frac{\Gamma(p+q)}{\Gamma(p)\Gamma(q)} x^{p-1} (1-x)^{q-1}$$

is given by

$$y = \frac{n}{2\pi} \int_{-\infty}^{\infty} e^{-inx\beta} \left\{ \frac{M_{\frac{1}{2}}^x(p-q), \frac{1}{2}(p+q-1)}{(-1\beta)_{\frac{1}{2}}(p+q)} \frac{(-1\beta)}{-} \right\}^n d\beta$$

where  $-\frac{1}{2} < \bar{x} < \frac{1}{2}$

the origin of this distribution being at the centre of the range, where  $M_{k,m}(z)$  is the confluent hypergeometric function defined by

$$M_{k,m}(z) = z^{\frac{1}{2}+m} e^{-\frac{1}{2}z} \left\{ 1 + \frac{\left(\frac{1}{2} + m - k\right)z}{1!(2m+1)} + \frac{\left(\frac{1}{2} + m - k\right)\left(\frac{3}{2} + m - k\right)z^2}{2!(2m+1)(2m+2)} + \dots \right\}$$

This will be true for all values of  $p$  and  $q$  admissible in Type I distributions provided that the integral involved is zero when  $x$  is greater than  $\frac{1}{2}$  or less than  $-\frac{1}{2}$ . This has only been proved for  $p, q$  positive integers when  $p$  and  $q$  are unequal but, in a previous paper, for all admissible values of  $p$  and  $q$  when  $p$  and  $q$  are equal. It is presumably true for all admissible values of  $p$  and  $q$  equal or unequal, but the development of the expression in this paper is confined to the case where  $p$  and  $q$  are positive integers.

(ii) When  $p$  and  $q$  are positive integers the above expression is integrable and the distribution of means has been shown to consist of arcs of parabolae of degree  $n(p+q-1)-1$  joining at the points

$$x = 0, \pm \frac{1}{n} \pm \frac{2}{n}, \dots \pm \frac{n-2}{2n} \quad \text{when } n \text{ is even}$$

and  $\bar{x} = \pm \frac{1}{2n}, \pm \frac{3}{2n}, \pm \frac{5}{2n}, \dots \pm \frac{n-2}{2n}$  when  $n$  is odd.

and having contact of order not less than  $nt-2$ , at the above points,  $t$  being the smaller of  $p$  and  $q$ .

The cases  $p = 1, 2, 3, 4$ ;  $q = 1, 2, 3, 4$ ;  $n = 2, 3, 4$  have been considered in detail.

It is interesting to note that these cases, when  $p$  or  $q$  is unity, include certain transitional  $J$  types of population with a finite ordinate at one end of the range and that the distribution of means of these types are curves with a zero ordinate at both ends of the range.

(iii) The region in which  $p$  or  $q$  or both lie between 0 and 1, that is in the region of the  $J$  and  $U$  curves has not so far been explored. For  $p = q = \frac{1}{2}$ , a symmetrical  $U$  curve, the distribution of means is given by

$$y = \frac{2}{2\pi} \int_{-\infty}^{\infty} \left\{ J_0 \left( \frac{\beta}{2} \right) \right\}^n \cos(n\bar{x}\beta) d\beta$$

an expression which has not so far been developed in a convenient form.

(iv) The first four moments of the distribution of means have been obtained and higher ones could be obtained if desired. The form of  $B_1$  and  $B_2$  shows of course that the distributions tend to the normal type as the size of sample becomes large and that this approach is rapid in the case of the symmetrical Type II curves.

## PART II.

### THE DISTRIBUTION OF THE MEANS OF SAMPLES FROM PEARSON'S TYPE VII FOR INTEGRAL VALUES OF $m$ .

#### (A) *The General Solution.*

In the relation (1)

$$\left( \int_a^b f(x) e^{\alpha x} dx \right)^n = \int_{na}^{nb} \psi(x) e^{\alpha x} dx$$

where  $y = f(x)$  is the original frequency distribution with range from  $x = a$  to  $x = b$  and  $Y = \psi(x)$  the distribution of means, we now put  $\alpha = i\beta$

and 
$$f(x) = \frac{\Gamma(m)}{\Gamma\left(m - \frac{1}{2}\right) \Gamma\left(\frac{1}{2}\right)} \frac{1}{(1+x^2)^m}$$

the range being from  $-\infty$  to  $\infty$ , obtaining

$$\left\{ \frac{\Gamma(m)}{\Gamma\left(m - \frac{1}{2}\right) \Gamma\left(\frac{1}{2}\right)} \int_{-\infty}^{\infty} \frac{e^{i\beta x}}{(1+x^2)^m} dx \right\}^n = \int_{-\infty}^{\infty} \psi(x) e^{i\beta x} dx$$

But

$$\int_{-\infty}^{\infty} \frac{e^{i\beta x}}{(1+x^2)^m} dx = \int_{-\infty}^{\infty} \frac{\cos \beta x}{(1+x^2)^m} dx + i \int_{-\infty}^{\infty} \frac{\sin \beta x}{(1+x^2)^m} dx$$

and the latter integral is zero because  $\sin \beta x$  is an odd function of  $x$ .

Thus the relation (1) may in this case be written

$$F(i\beta) = \left\{ \int_{-\infty}^{\infty} \frac{\Gamma(m)}{\Gamma\left(m - \frac{1}{2}\right) \Gamma\left(\frac{1}{2}\right)} \frac{\cos \beta x}{(1+x^2)^m} dx \right\}^n = \int_{-\infty}^{\infty} \psi(x) \cos \beta x dx \quad \dots (53)$$

whence it follows by FOURIER's Integral Theorem that

$$\psi(x) = \frac{1}{\pi} \int_0^{\infty} F(i\beta) \cos \beta x d\beta \quad \dots (54)$$

Now it may be shown by contour integration that if  $m$  is an integer

$$\int_{-\infty}^{\infty} \frac{\cos \beta x}{(1+x^2)^m} dx = \frac{\pi e^{-\beta}}{2^{2m-2}} \left\{ \sum_{r=0}^{m-1} \frac{m(m+1)\dots(m+r-1)}{r!(m-1-r)!} (2\beta)^{m-r-1} \right\}^*$$

(\*) This may be proved as follows:—

Consider  $\int_{-\infty}^{\infty} \frac{e^{i\beta x}}{(1+x^2)^m} dx$  where  $m$  is a positive integer. First suppose

$$\begin{aligned}
&= \frac{\pi e^{-\beta}}{2^{2m-2}} \left\{ \frac{(2\beta)^{m-1}}{(m-1)!} + \frac{m(2\beta)^{m-2}}{1!(m-2)!} + \dots + \right. \\
&+ \frac{m(m+1)\dots(m+r-1)}{r!(m-1-r)!} (2\beta)^{m-r-1} \dots + \left. \frac{m(m+1)\dots(2m-2)}{(m-1)!} \right\} \\
&\dots (55)
\end{aligned}$$

$\beta > 0$  and consider  $\int_c \frac{e^{\beta z} dz}{\left(1 + \frac{z^2}{a^2}\right)^m}$ ,  $c$  being a semi-circular contour  $BAC$

of radius  $R$  and centre at the origin.

The only singularity of the integrand within the contour is at  $P(z = ia)$   
 $a > 0$ .

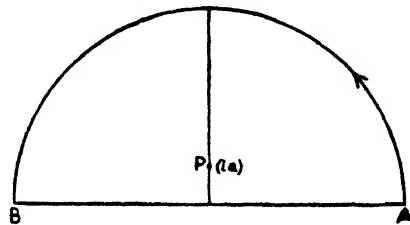


FIG. 2.

Hence 
$$\int_c \frac{e^{\beta z} dz}{\left(1 + \frac{z^2}{a^2}\right)^m} = 2\pi i \text{ (residue at } P).$$

Consider 
$$\int_{ACB} \frac{e^{\beta z} dz}{\left(1 + \frac{z^2}{a^2}\right)^m} \quad \text{Let } z = Re^{i\theta} \text{ on } ACB.$$

Then on  $ACB$

$$\left| \frac{e^{\beta z}}{\left(1 + \frac{z^2}{a^2}\right)^m} \right| < \frac{e^{-R\beta \sin \theta}}{\left(\frac{R^2}{a^2} - 1\right)^m} \leq \frac{Ae^{-R\beta \sin \theta}}{R}$$

where  $A$  is a positive constant, for sufficiently large values of  $R$ .

Hence 
$$\int_{ACB} \frac{e^{\beta z} dz}{\left(1 + \frac{z^2}{a^2}\right)^m} \rightarrow 0 \quad \text{as } R \rightarrow \infty$$

Thus

$$F(i\beta) = \left\{ \frac{\Gamma(m)}{\Gamma\left(m - \frac{1}{2}\right) \Gamma\left(\frac{1}{2}\right)} \right\}^n \left\{ \int_{-\infty}^{\infty} \frac{\cos \beta x dx}{\left(1 + x^2\right)^m} \right\}^n \\ = e^{-n\beta} \sum_{r=0}^{n(m-1)} A_r \beta^r$$

And

$$\int_{-\infty}^{\infty} \frac{e^{i\beta x} dx}{\left(1 + \frac{x^2}{a^2}\right)^m} = 2\pi i \quad (\text{residue at } P)$$

To calculate the residue we put  $z = ia + t$  in  $\frac{e^{i\beta z}}{\left(1 + \frac{z^2}{a^2}\right)^m}$  and pick out

the coefficient of  $\frac{1}{t}$ .

Now

$$\frac{e^{i\beta z}}{\left(1 + \frac{z^2}{a^2}\right)^m} = \frac{a^{2m} e^{-a\beta} e^{i\beta t}}{(2ia)^m t^m \left(1 + \frac{t}{2ia}\right)^m} \\ = \frac{a^m e^{a\beta} e^{i\beta t}}{(2i)^m t^m} \left(1 - \frac{mt}{2ia} + \dots + (-1)^s \frac{m(m-1)\dots(m+s-1)}{(2ia)^s s!} t^s + \dots\right)$$

whence the integral is equal to

$$\frac{\pi}{2^{2m-2}} a e^{-a\beta} \left\{ \frac{(2a\beta)^{m-1}}{(m-1)!} + \dots + \frac{m(m-1)\dots(m+s-1)}{s!(m-1-s)!} (2a\beta)^{n-s-1} + \dots \right. \\ \left. + \dots + \frac{m(m+1)\dots(2m-2)}{(m-1)!} \right\}$$

If  $\beta$  were negative we should have to take the contour below the real axis and we should have

$$\int_{-\infty}^{\infty} \frac{e^{i\beta x} dx}{\left(1 + \frac{x^2}{a^2}\right)^m} = -2\pi i \quad (\text{residue at } z = -ia) \\ = \frac{\pi(-1)^{m-1}}{2^{2m-2}} a e^{a\beta} \left\{ \frac{(2a\beta)^{m-1}}{(m-1)!} - \frac{m(2a\beta)^{m-2}}{1!(m-2)!} + \dots \right. \\ \left. + \dots + \frac{(-1)^s m(m+1)\dots(m+s-1)}{s!(m-1-s)!} (2\beta)^{m-s-1} \right. \\ \left. + \dots + \frac{(-1)^{m-1} m(m+1)\dots(2m-2)}{(m-1)!} \right\}$$

where the coefficients  $A_r$  may be determined by substituting expression (55) for the integral in  $F(i\beta)$  and expanding the multinomial. Thus

$$\begin{aligned}\psi(x) &= \frac{1}{\pi} \int_0^\infty \sum_{r=0}^{n(m-1)} A_r \beta^r e^{-n\beta} \cos \beta x d\beta = \\ &= \frac{1}{\pi} \sum_{r=0}^{n(m-1)} A_r \int_0^\infty \beta^r e^{-n\beta} \cos \beta x d\beta \quad \dots\dots(56)\end{aligned}$$

Now it is known that when  $a, n$  are positive

$$\int_0^\infty e^{-ax} x^{n-1} \cos bx dx = \frac{\Gamma(n) \cos n\vartheta}{r^n}$$

where

$$r = (a^2 + b^2)^{\frac{1}{2}} \quad \vartheta = \tan^{-1} \frac{b}{a} \quad (*)$$

Thus

$$\psi(x) = \frac{1}{\pi} \sum_{r=0}^{n(m-1)} A_r \frac{\Gamma(r+1) \cos \left\{ (r+1) \tan^{-1} \frac{x}{n} \right\}}{(n^2 + x^2)^{\frac{r+1}{2}}}$$

The two results may be combined in the formula

$$\frac{\pi a^{-\frac{1}{2}} e^{-a|\beta|}}{2^{\frac{1}{2}} m^{-\frac{1}{2}}} \left\{ \sum_{s=0}^{m-1} \frac{m(m+1)}{s!(m-1-s)!} (m+s-1) \left[ 2a|\beta| \right]^{m-s-1} \right\}$$

We may also write (whether  $m$  is an integer or not)

$$\int_{-\infty}^{\infty} \frac{\cos \beta x dx}{(1+x^2)^m} = \int_{-\infty}^{\infty} \frac{e^{i\beta x} dx}{(1+x^2)^m} = \frac{\pi^{\frac{1}{2}} \beta^{m-\frac{1}{2}}}{2^{m-\frac{3}{2}} \Gamma(m)} \frac{K_{m-\frac{1}{2}}(\beta)}{\cos\left(m-\frac{1}{2}\right)\pi}$$

where  $K_{m-\frac{1}{2}}(\beta)$  is a modified BESSEL Function of the second kind (See (15) pp. 367 and 377). This might have been expected by analogy with Type II previously discussed.

(\*) (17) pag. 471.

and the distribution of means is given by

$$y = \frac{n}{\pi} \sum_{r=0}^{n(m-1)} \frac{A_r \Gamma(r+1) \cos \left\{ (r+1) \tan^{-1} \bar{x} \right\}}{n^{r+1} (1 + \bar{x}^2)^{\frac{r+2}{2}}}$$

$$= \frac{n}{\pi} \sum_{r=0}^{n(m-1)} \frac{A_r \Gamma(r+1) \cos \left\{ (r+1) \cos^{-1} \frac{1}{\sqrt{1 + \bar{x}^2}} \right\}}{n^{r+1} (1 + \bar{x}^2)^{\frac{r+2}{2}}} \dots\dots (57)$$

Now in virtue of the well known relations

$$\cos s \vartheta$$

$$= (-1)^{\frac{s}{2}} \left\{ 1 + \sum_{r=1}^s \frac{(-1)^r s^2 (s^2 - 2^2) \dots (s^2 - 2r + 2^2) \cos^{2r} \vartheta}{(2r+1)!} \right\}$$

if  $s$  is an even integer

and

$$\cos s \vartheta = (-1)^{\frac{s-1}{2}} \left\{ s \cos \vartheta + \sum_{r=1}^{\frac{s-1}{2}} s (s^2 - 1^2) \frac{(s^2 - 3^2)}{(r+1)!} \dots (s^2 - (2r-1)^2) \cos^{2r+1} \vartheta \right\} \dots (58)$$

it follows at once that (57) may be put in a form which involves only negative integral powers of  $(1 + \bar{x}^2)$  The form of the distribution of means is thus the sum of a number of Type VII curves

### (B) *Particular Cases.*

We will now consider some particular cases

(i)  $m = 1$

The population curve is  $y = \frac{1}{\pi} \frac{1}{1 + x^2}$

Here 
$$\left[ \frac{\Gamma(m)}{\Gamma\left(m - \frac{1}{2}\right) \Gamma\left(\frac{1}{2}\right)} \right]^n = \frac{1}{\pi^n}$$

Hence  $A_0 = 1$   $A_r = 0$  for  $r > 0$ .

Therefore the distribution of means is given by

$$y = \frac{n}{\pi} \frac{\cos \tan^{-1} \bar{x}}{n (1 + \bar{x}^2)^{\frac{1}{2}}} = \frac{1}{\pi} \frac{1}{1 + \bar{x}^2}$$



Thus the distribution of means in random samples of *any size* from the curve

$$y = \frac{1}{\pi} \frac{1}{(1+x^2)} \quad \dots\dots (59)$$

is the curve itself. This remarkable fact has already been pointed out by R. A. FISHER (1<sub>4</sub>) (\*) and could not of course have arisen, were not the standard deviation of the original population and hence the standard deviation of the mean, infinite.

(ii)  $m = 2$

The population curve is  $y = \frac{2}{\pi} \frac{1}{(1+x^2)^2}$

$$\begin{aligned} \text{Here} \quad F(i\beta) &= \left\{ \pi e^{-\beta} (\beta + 1) \right\}^n \\ &= \left\{ \Gamma\left(\frac{3}{2}\right) \Gamma\left(\frac{1}{2}\right) \right\}^n \\ &= e^{-n\beta} (\beta + 1)^n \\ &= \sum_{r=0}^n {}^nC_r \beta^r e^{-n\beta} \end{aligned}$$

Hence  $A_r = {}^nC_r$ ,

and the distribution of means is given by

$$y = \frac{1}{\pi} \sum_{r=0}^n \frac{n(n-1)\dots(n-r+1) \cos(r + 1 \tan^{-1} \bar{x})}{n^r (1 + \bar{x}^2)^{\frac{r+1}{2}}} \quad \dots\dots (60)$$

Putting  $n = 2, 3, 4$  in succession we obtain

*Samples of 2.*

$$\begin{aligned} y &= \frac{1}{\pi} \left\{ \frac{\cos \tan^{-1} \bar{x}}{(1 + \bar{x}^2)^{\frac{1}{2}}} + \cos \frac{(2 \tan^{-1} \bar{x})}{(1 + \bar{x}^2)} + \frac{\cos (3 \tan^{-1} \bar{x})}{4 (1 + \bar{x}^2)^{\frac{3}{2}}} \right\} \\ &= \frac{1}{\pi} \left\{ \frac{5}{4 (1 + \bar{x}^2)^2} + \frac{1}{(1 + \bar{x}^2)^3} \right\} \quad \dots\dots (61) \end{aligned}$$

---

(\*) Questa curva di distribuzione era già stata studiata dal CAUCHY nel 1853, e la proprietà accennata nel testo era anche precedentemente stata segnalata dal POISSON. (cfr. (13), p. 179). (*Nota della Redazione*).

*Samples of 3.*

$$\begin{aligned}
 y &= \frac{1}{\pi} \left\{ \frac{\cos \tan^{-1} \bar{x}}{(1 + \bar{x}^2)^{\frac{1}{2}}} + \frac{\cos (2 \tan^{-1} \bar{x})}{(1 + \bar{x}^2)} + \frac{2}{3} \frac{\cos (3 \tan^{-1} \bar{x})}{(1 + \bar{x}^2)^{\frac{3}{2}}} + \right. \\
 &\quad \left. + \frac{2}{9} \frac{\cos (4 \tan^{-1} \bar{x})}{(1 + \bar{x}^2)^2} \right\} \\
 &= \frac{2}{9\pi} \left\{ \frac{1}{(1 + \bar{x}^2)^2} + \frac{4}{(1 + \bar{x}^2)^3} + \frac{8}{(1 + \bar{x}^2)^4} \right\} \dots\dots (62)
 \end{aligned}$$

*Samples of 4.*

$$\begin{aligned}
 y &= \frac{1}{\pi} \left\{ \frac{\cos \tan^{-1} \bar{x}}{(1 + \bar{x}^2)^{\frac{1}{2}}} + \frac{\cos (2 \tan^{-1} \bar{x})}{(1 + \bar{x}^2)} + \frac{3}{4} \frac{\cos (3 \tan^{-1} \bar{x})}{(1 + \bar{x}^2)^{\frac{3}{2}}} \right. \\
 &\quad \left. + \frac{3}{8} \frac{\cos (4 \tan^{-1} \bar{x})}{(1 + \bar{x}^2)^2} + \frac{3}{32} \frac{\cos (5 \tan^{-1} \bar{x})}{(1 + \bar{x}^2)^{\frac{5}{2}}} \right\} \\
 &= \frac{1}{32\pi} \left\{ \frac{4}{(1 + \bar{x}^2)^2} + \frac{15}{(1 + \bar{x}^2)^3} + \frac{36}{(1 + \bar{x}^2)^4} + \frac{48}{(1 + \bar{x}^2)^5} \right\}
 \end{aligned}$$

We shall now write down the distribution of means for samples of 2, 3, 4 up to  $p = 4$  and for samples of  $z$  up to  $p = 8$ . The process of reduction is in every case the same.

First  $\int_{-\infty}^{\infty} \frac{\cos \beta x}{(1 + x^2)^m} dx$  is calculated from (55), then by squaring,

cubing or raising to the 4th. power, as the case may be, and multiplying by

$\left\{ \frac{\Gamma(m)}{\Gamma\left(m - \frac{1}{2}\right) \Gamma\left(\frac{1}{2}\right)} \right\}^n$ ,  $F(i\beta)$  is obtained. This gives

the A's necessary for substitution in (57).

Then by means of the series for  $\cos s\theta$ , (57) is reduced to the simple form which involves only negative powers of  $(1 + \bar{x}^2)$ .

The values of  $F(i, \beta)$  are as follows :—

$m$	Value of $F(i, \beta)$
1	$e^{-n\beta}$
2	$e^{-n\beta} (1 + \beta)^n$
3	$e^{-n\beta} \left(1 + \beta + \frac{\beta^2}{3}\right)^n$
4	$e^{-n\beta} \left(1 + \beta + \frac{2}{5}\beta^2 + \frac{1}{15}\beta^3\right)^n$
5	$e^{-n\beta} \left(1 + \beta + \frac{3}{7}\beta^2 + \frac{2}{21}\beta^3 + \frac{1}{105}\beta^4\right)^n$
6	$e^{-n\beta} \left(1 + \beta + \frac{4}{9}\beta^2 + \frac{1}{9}\beta^3 + \frac{1}{63}\beta^4 + \frac{1}{945}\beta^5\right)^n$
7	$e^{-n\beta} \left(1 + \beta + \frac{5}{11}\beta^2 + \frac{28}{231}\beta^3 + \frac{2}{99}\beta^4 + \frac{1}{495}\beta^5 + \frac{1}{10395}\beta^6\right)^n$
8	$e^{-n\beta} \left(1 + \beta + \frac{66}{143}\beta^2 + \frac{55}{429}\beta^3 + \frac{10}{429}\beta^4 + \frac{2}{715}\beta^5 + \frac{4}{19305}\beta^6 + \frac{\beta^7}{135135}\right)^n$

..... (64)

The A's may be obtained from these results and the subsequent reductions though somewhat laborious are simple. The final results will now be given. They have been checked by verifying in each case by direct integration that the total frequency is unity.

(iii)  $m = 3$

The population curve is

$$y = \frac{8}{3\pi} \frac{1}{(1 + x^2)^3}$$

Samples of 2.

$$y = \frac{1}{\pi} \left\{ \frac{1}{6(1 + \bar{x}^2)^3} + \frac{2}{3(1 + \bar{x}^2)^4} + \frac{8}{3(1 + \bar{x}^2)^5} \right\} \dots\dots (65)$$

Samples of 3.

$$y = \frac{1}{\pi} \left\{ \frac{8}{243} \frac{1}{(1 + \bar{x}^2)^3} + \frac{304}{2187} \frac{1}{(1 + \bar{x}^2)^4} + \frac{1024}{2187} \frac{1}{(1 + \bar{x}^2)^5} + \right. \\ \left. + \frac{2560}{2187} \frac{1}{(1 + \bar{x}^2)^6} + \frac{5120}{2187} \frac{1}{(1 + \bar{x}^2)^7} \right\} \dots\dots (66)$$

Samples of 4.

$$y = \frac{1}{\pi} \left\{ \frac{1}{96} \frac{1}{(1 + \bar{x}^2)^3} + \frac{11}{256} \frac{1}{(1 + \bar{x}^2)^4} + \frac{67}{512} \frac{1}{(1 + \bar{x}^2)^5} + \right. \\ \left. + \frac{65}{192} \frac{1}{(1 + \bar{x}^2)^6} + \frac{25}{32} \frac{1}{(1 + \bar{x}^2)^7} + \frac{35}{24} \frac{1}{(1 + \bar{x}^2)^8} + \frac{35}{18} \frac{1}{(1 + \bar{x}^2)^9} \right\} \\ \dots\dots (67)$$

(iv)  $m = 4$

The population curve is

$$y = \frac{16}{5\pi} \frac{1}{(1 + \bar{x}^2)^4}$$

Samples of 2.

$$y = \frac{1}{\pi} \left\{ \frac{1}{20} \frac{1}{(1 + \bar{x}^2)^4} + \frac{6}{25} \frac{1}{(1 + \bar{x}^2)^5} + \frac{4}{5} \frac{1}{(1 + \bar{x}^2)^6} + \frac{16}{5} \frac{1}{(1 + \bar{x}^2)^7} \right\} \\ \dots\dots (68)$$

Samples of 3.

$$y = \frac{1}{164025\pi} \left\{ \frac{720}{(1 + \bar{x}^2)^4} + \frac{3712}{(1 + \bar{x}^2)^5} + \frac{12544}{(1 + \bar{x}^2)^6} + \frac{37888}{(1 + \bar{x}^2)^7} + \right. \\ \left. + \frac{100352}{(1 + \bar{x}^2)^8} + \frac{229376}{(1 + \bar{x}^2)^9} + \frac{458752}{(1 + \bar{x}^2)^{10}} \right\} \dots\dots (69)$$

Samples of 4.

$$\frac{1}{2048000\pi} \left\{ \frac{1600}{(1 + \bar{x}^2)^4} + \frac{8160}{(1 + \bar{x}^2)^5} + \frac{26760}{(1 + \bar{x}^2)^6} + \frac{73767}{(1 + \bar{x}^2)^7} + \frac{185724}{(1 + \bar{x}^2)^8} \right. \\ \left. + \frac{438816}{(1 + \bar{x}^2)^9} + \frac{983808}{(1 + \bar{x}^2)^{10}} + \frac{2016000}{(1 + \bar{x}^2)^{11}} + \frac{3548160}{(1 + \bar{x}^2)^{12}} + \frac{4730880}{(1 + \bar{x}^2)^{13}} \right\} \\ \dots\dots (70)$$

We add also the distributions for samples of 2 for the cases  $m = 5, 6, 7, 8$ .

*Samples of 2.*

(v)  $m = 5$ .

The population curve is

$$y = \frac{128}{35\pi} \frac{1}{(1 + \bar{x}^2)^5}$$

The distribution of means is

$$y = \frac{1}{490\pi} \left\{ \frac{7}{(1 + \bar{x}^2)^5} + \frac{40}{(1 + \bar{x}^2)^6} + \frac{144}{(1 + \bar{x}^2)^7} + \frac{448}{(1 + \bar{x}^2)^8} + \frac{1792}{(1 + \bar{x}^2)^9} \right\} \dots\dots (71)$$

(vi)  $m = 6$

The population curve is

$$y = \frac{256\pi}{63} \frac{1}{(1 + \bar{x}^2)^6}$$

The distribution of means is

$$y = \frac{1}{756\pi} \left\{ \frac{3}{(1 + \bar{x}^2)^6} + \frac{20}{(1 + \bar{x}^2)^7} + \frac{80}{(1 + \bar{x}^2)^8} + \frac{256}{(1 + \bar{x}^2)^9} + \frac{768}{(1 + \bar{x}^2)^{10}} + \frac{3072}{(1 + \bar{x}^2)^{11}} \right\} \dots\dots (72)$$

(vii)  $m = 7$

The population curve is

$$y = \frac{1024}{231\pi} \frac{1}{(1 + \bar{x}^2)^7}$$

The distribution of means is

$$y = \frac{1}{30492\pi} \left\{ \frac{33}{(1 + \bar{x}^2)^7} + \frac{252}{(1 + \bar{x}^2)^8} + \frac{1120}{(1 + \bar{x}^2)^9} + \frac{3840}{(1 + \bar{x}^2)^{10}} + \frac{11520}{(1 + \bar{x}^2)^{11}} + \frac{33792}{(1 + \bar{x}^2)^{12}} + \frac{135168}{(1 + \bar{x}^2)^{13}} \right\} \dots\dots (73)$$

(viii)  $m = 8$

The population curve is

$$y = \frac{2048}{429\pi} \frac{1}{(1 + \bar{x}^2)^8}$$

The distribution of means is

$$y = \frac{1}{490776\pi} \left\{ \frac{143}{(1+\bar{x}^2)^8} + \frac{1232}{(1+\bar{x}^2)^9} + \frac{6048}{(1+\bar{x}^2)^{10}} + \frac{22400}{(1+\bar{x}^2)^{11}} + \right. \\ \left. + \frac{70400}{(1+\bar{x}^2)^{12}} + \frac{202752}{(1+\bar{x}^2)^{13}} + \frac{585728}{(1+\bar{x}^2)^{14}} + \frac{2342912}{(1+\bar{x}^2)^{15}} \right\} \dots\dots (74)$$

(C) *Moments of the Distribution of Means.*

The moments of the Type VII curve

$$y = \frac{\Gamma(m)}{\Gamma\left(m - \frac{1}{2}\right) \Gamma\left(\frac{1}{2}\right)} \frac{1}{(1+x^2)^m}$$

have been shown by PEARSON to be given by

$$\mu_{2s+1} = 0 \\ \mu_{2s} = \frac{\left(s - \frac{1}{2}\right) \left(s - \frac{3}{2}\right) \dots\dots\dots \frac{1}{2}}{\left(m - \frac{3}{2}\right) \left(m - \frac{5}{2}\right) \dots \left(m - \frac{s}{2} - \frac{1}{2}\right)} \left\{ \dots\dots (75) \right.$$

whence we find

$$\mu_2 = \frac{1}{2m-3} \\ \mu_4 = \frac{3}{(2m-3)(2m-5)}$$

Whence using the method of p. 38 we find for the distribution of means

$$M_2 = \frac{1}{n(2m-3)} \\ M_4 - 3M_2^2 = \frac{6}{n^3(2m-3)^2(2m-5)} \left\{ \dots\dots (76) \right.$$

Whence

$$M_4 = \frac{1}{n^3(2m-3)^2} \left\{ 3 + \frac{6}{n(2m-5)} \right\} \dots\dots (77)$$

and we have

$$\left. \begin{aligned} B_1 &= 0 \\ B_2 &= 3 + \frac{6}{n(2m-5)} \end{aligned} \right\} \dots\dots (78)$$

so that the approach to normality with increasing size of sample is rapid.

#### (D) Summary to Part II.

(i). The distribution of the means of random samples of  $n$  from a population, supposed indefinitely large, of the Type

$$y = \frac{\Gamma(m)}{\Gamma\left(m - \frac{1}{2}\right) \Gamma\left(\frac{1}{2}\right)} \frac{1}{(1+x^2)^m}$$

is given for integral values of  $m$  by

$$y = \frac{n^{n(m-1)}}{\pi} \sum_{r=0}^{n(m-1)} \frac{A_r \Gamma(r+1) \cos\{(r+1) \tan^{-1} \bar{x}\}}{n^{r+1} (1+\bar{x}^2)^{\frac{r+1}{2}}}$$

(ii). This may be expressed in a form which involves only negative integral powers of  $\frac{1}{(1+\bar{x}^2)}$  the lowest power of  $\frac{1}{(1+\bar{x}^2)}$  which occurs being the  $m^{\text{th}}$  and the highest the  $\{n(m-1) + 1\}^{\text{th}}$ .

(iii). The cases  $m=1$  for all values of  $n$ ;  $m=2, 3, 4$ ,  $n=2, 3, 4$ ; and  $m=5, 6, 7, 8$ ,  $n=2$  have been considered in detail.

(iv). For non-integral values of  $m$  the distribution is given by

$$y = \frac{1}{\pi} \left\{ 2^{m-\frac{3}{2}} \Gamma\left(m - \frac{1}{2}\right) \right\}^{-n} \int_0^\pi \left\{ \frac{\beta^{m-\frac{1}{2}} K_{m-\frac{1}{2}}(\beta)}{\cos\left(m - \frac{1}{2}\right)\pi} \right\}^n \cos \beta x d\beta$$

where  $K_{m-\frac{1}{2}}(\beta)$  is a modified BESSEL function of the second kind, but this expression has not as yet been dealt with.

(v). The moments of the distribution of means are easily written down, and the approach to normality with increasing size of sample is rapid.

## GENERAL SUMMARY.

(1) The present method of approach has now led to a complete solution of the problem of the distribution of means of random samples from populations of normal type and of PEARSON'S Type III  $\left[ y = y_0 e^{-\frac{p x}{a}} \left( 1 + \frac{x}{a} \right)^p \right]$ . The exponential curve may for this purpose be regarded as a particular case of Type III. See (14) p. 228.

(2) This method has also led to general expressions for the distribution of means of populations of Pearson's Types II, VII and I; but these expressions can only be integrated and reduced to an explicit form for integral values of the indices involved.

(3) It seems probable that further research on these lines will throw light on the distribution of means of the remaining Pearsonian Types also; but perhaps the main interest of the method employed is to show how, once we are given the moments of the sampling distribution of any moment function, we may proceed from these to the actual distribution itself, provided that the integral solution can be put into explicit form. This is undoubtedly the hardest part of the problem, but the fact that somewhat unpromising analytical forms have been dealt with here and that the recent comprehensive work of R. A. FISHER has made the moments of moment functions far more available than hitherto, opens up the prospect that the exact sampling distributions of many moment functions will ultimately be obtained.

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XXIV.—The General Form of the Orthogonal Polynomials for Simple Series, with Proofs of their Simple Properties. By F. E. Allan, M.A. (Melbourne), Statistical Department, Rothamsted Experimental Station, Harpenden, Herts. *Communicated by Professor E. T. WHITTAKER, F.R.S.*

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INTRODUCTORY.

IN *Statistical Methods for Research Workers* R. A. Fisher (1) has given a numerical method of curve fitting by means of orthogonal polynomials. It is proposed here to show how the properties of these polynomials and a general formula for them may be developed from the properties of their terminal differences, thus providing general proofs of the properties utilised in the numerical method.

PRINCIPLE OF CURVE FITTING BY ORTHOGONAL POLYNOMIALS.

Suppose that  $y$  is the dependent variate whose values  $y_1, y_2, \dots, y_n$  have been observed at the points  $x_1, x_2, \dots, x_n$  of the independent variate  $x$ , and that it is desired to fit a polynomial of degree  $r$  to these observations.

Clearly we can express any polynomial of the  $r$ th degree in the form

$$\sum_{p=0}^r A_p \xi_p(x)$$

where  $\xi_p(x)$  is an arbitrarily chosen polynomial of degree  $p$ , and the coefficients  $A_0, A_1$ , etc., are suitably chosen.

In curve fitting by orthogonal polynomials we write the equation in the form

$$Y = A_0 + A_1 \xi_1 + \dots + A_r \xi_r$$

and choose for the  $\xi$ 's those polynomials which are determined successively apart from a constant factor, by the equations

$$\begin{aligned} \xi_0 &= 1 \\ S(\xi_0 \xi_1) &= 0 \\ S(\xi_0 \xi_2) &= 0 \\ S(\xi_1 \xi_2) &= 0 \end{aligned}$$

and so on.

In general  $\xi_s$  is formed from the preceding polynomials by the system of orthogonal equations

$$S(\xi_p \xi_s) = 0$$

where  $p=0, 1, 2, \dots, (s-1)$ , and  $S$  denotes summation over the given series of values  $x_1, x_2, \dots, x_n$  of  $x$ .

#### THE ADVANTAGES OF EMPLOYING AN ORTHOGONAL SYSTEM.

To evaluate the coefficients  $A_1, A_2, \dots, A_r$  we form the sum

$$S\left[(y - Y)^2\right] = S\left[\left\{y - \sum_{s=0}^r A_s \xi_s\right\}^2\right] \quad . \quad . \quad . \quad . \quad (I)$$

and choose the coefficients so that this sum will be a minimum. The condition for this is that for all integral values of  $p$  from 0 to  $r$

$$S\left[\left\{y - \sum_{s=0}^r (A_s \xi_s)\right\} \xi_p\right] = 0. \quad . \quad . \quad . \quad . \quad (II)$$

But, on account of the orthogonal conditions,

$$S\left[\sum_{s=0}^r (A_s \xi_s) \xi_p\right]$$

becomes simply

$$S(A_p \xi_p^2)$$

and the system of simultaneous equations (II) reduces to direct equations for the coefficients of the form

$$S(A_p \xi_p^2) = S(y \xi_p)$$

or simply

$$A_p = \frac{S(y \xi_p)}{S(\xi_p^2)} \quad . \quad . \quad . \quad . \quad . \quad (III)$$

Thus we are able, by the use of orthogonal functions, to determine each coefficient directly, independently of the other terms fitted. It is therefore possible to extend the process, by including new terms of higher degree, without affecting the coefficients already calculated.

In testing the goodness of fit we need to evaluate the expression (I) for the sum of the squares of the residuals. With the value found for  $A_p$  this sum becomes

$$S(y^2) - \sum_{s=0}^r \left[ S(A_s^2 \xi_s^2) \right] \quad . \quad . \quad . \quad . \quad (IV)$$

and clearly this is reduced for each term  $A_s \xi_s$  by the sum  $S\{(A_s \xi_s)^2\}$  taken over points of the series. The residual variance is got by dividing the expression (IV) by the number of degrees of freedom left after the  $r+1$  terms have been fitted.

## ANNUAL SERIES.

The orthogonal polynomials can be used with greatest advantage in problems relating to annual series where the increments of time are equal. The form of the polynomials that satisfy orthogonal conditions for  $n$  given values of  $x$  naturally depends on the distribution of these values, and for annual series we can obtain fixed forms for them in which the coefficients of  $x$  are certain functions of  $n$ , the number of observations. It would be possible for special distributions to determine the form, but obviously the most valuable application of the polynomials is to those cases in which a standard form can be used. Tchebycheff (2), who first studied these orthogonal polynomials, found explicit expressions in the simple case up to the fifth degree. He gave the results

$$\begin{aligned}\xi_1 &= 2x \\ \xi_2 &= 12x^2 - (n^2 - 1) \\ \xi_3 &= 120x^3 - 6(3n^2 - 7)x \\ \xi_4 &= 1680x^4 - 120(3n^2 - 13)x^2 + 9(n^2 - 1)(n^2 - 9) \\ \xi_5 &= 30240x^5 - 8400(n^2 - 7)x^3 + 30(15n^4 - 230n^2 + 407)x\end{aligned}$$

where  $x$  is measured from the mid-point of the series. These were found by means of the reduction formula

$$\xi_r = 2(2r - 1)x\xi_{r-1} - (r - 1)^2[n^2 - (r - 1)^2]\xi_{r-2}.$$

The proof of this relation is given in Tchebycheff's papers (2, pp. 541-560), but it is too complicated to reproduce here. Later we shall see how the result can be very simply inferred from the orthogonal relation with the help of the terminal difference properties.

Tchebycheff's methods were very involved, and much later the subject was treated independently by Esscher (3), who showed how to get the first four expressions, by Jordan (5) and by R. A. Fisher (4), who gave explicit expressions for the first five polynomials, differing from Tchebycheff's form only in respect of a numerical factor, the coefficient of  $x^r$  in  $\xi_r$  being unity in Fisher's formula and  $\frac{(2r)!}{r!}$  in Tchebycheff's system. Since the coefficient of  $\xi_r$  in the function  $Y$  is

$$A_r = \frac{S(y\xi_r)}{S(\xi_r^2)}$$

it is apparent that this factor can be absorbed in the coefficient and cannot affect the value of the function.

# METHODS OF DETERMINING THE POLYNOMIALS.

There have been two different procedures adopted—one, the algebraic method of finding general expressions which, with appropriate substitutions, could be applied to any set of data; and the other, the numerical method established by Fisher, in which for any special case the values of the function can be built up by a summation process from terminal differences.

## ALGEBRAIC PROCESS.

The direct method of finding algebraic forms for the polynomials is to determine them from the orthogonal equations in terms of moments of the distribution. Write

$$\xi_r = x^r + \sum_{s=r-1}^0 a_s x^s \quad . \quad . \quad . \quad . \quad (V)$$

for the polynomial of degree  $r$ . The orthogonal condition

$$S(\xi_s \xi_r) = 0$$

is equivalent to

$$S(x^s \xi_r) = 0 \quad . \quad . \quad . \quad . \quad . \quad (VI)$$

for all integral values of  $s$  from 0 to  $r-1$ . If in equation (VI) we give  $s$  successively the values 0 to  $(r-1)$ , and if we substitute for  $\xi_r$  from equation (V), then we get the following  $(r+1)$  equations in the unknowns  $a_0, a_1, \dots, a_{r-1}$  :—

$$\begin{aligned} \xi_r - x^r + a_{r-1}x^{r-1} + a_{r-2}x^{r-2} + \dots \quad a_0 &= 0 \\ \mu_r + a_{r-1}\mu_{r-1} + a_{r-2}\mu_{r-2} + \dots \quad a_0 &= 0 \\ \mu_{r+1} + a_{r-1}\mu_r + a_{r-2}\mu_{r-1} + \dots \quad a_0\mu_1 &= 0 \end{aligned}$$

and so on, where  $\mu_s$  is the  $s$ th moment of  $x$  about any origin.

From these  $(r+1)$  equations we deduce the equation for  $\xi_r$  at once in the form

$$\begin{vmatrix} x^r - \xi_r & x^{r-1} & x^{r-2} & & 1 \\ \mu_r & \mu_{r-1} & \mu_{r-2} & & 1 \\ \mu_{r+1} & \mu_r & \mu_{r-1} & & \mu_1 \\ . & . & . & . & . \\ . & . & . & . & . \\ \mu_{2r-1} & \mu_{2r-2} & \mu_{2r-3} & \dots & \mu_{r-1} \end{vmatrix} = 0.$$

In this way the coefficients of powers of  $x$  in  $\xi_r$  are each given as the ratio of two moment determinants, and their values can be at once calculated for any set of values  $x_1, x_2, \dots, x_n$ . In the simplified case where the intervals of  $x$  are equal and can be taken as unity, if we take the mean

as origin, then the odd moments vanish, and  $\mu_{2s}$  is a constant multiple of the sum of the  $(2s)$ th powers of the numbers differing by 1 and going from  $\frac{n-1}{2}$  to 0 or  $\frac{1}{2}$ , according as  $n$  is odd or even. For polynomials up to the fifth or sixth degree this method is quite satisfactory; but when higher power terms are wanted the work becomes very heavy, and we need to look for some other way of finding algebraic expressions for the polynomials. It is to be noted that while Tchebycheff's reduction formula applies only to problems in which  $x$  is given at equal intervals, the equation in terms of moments is general.

#### THE NUMERICAL METHOD.

We shall consider from now onwards only the simplified case in which the intervals between given points of the series are unity. The numerical procedure depends on knowing the values of the end differences of the polynomials and building up the function  $Y$  from them; for if we fit  $(r+1)$  terms, the  $r$ th difference is constant and equal to  $\Delta_r$ , and by addition to the  $(r-1)$ th terminal difference we can find all the  $(r-1)$ th differences, and continuing the process back we arrive ultimately at the values of the function itself at points of the series. The same method of building from terminal differences will give the quickest means of finding the actual values of any one of the orthogonal polynomials at points of the series, when these are desired.

#### THE TERMINAL DIFFERENCES.

It is the purpose here to extend the process and obtain a general form for the polynomial of degree  $r$  from its central differences, these being built up from the terminal differences. It will be necessary first of all to establish the form of the terminal differences, and at the same time we can prove certain other useful properties of the polynomials, including Tchebycheff's reduction formula.

If  $\Delta^m$  represents the  $m$ th terminal difference of  $\xi_r$ , we can write

$$\xi_r = \Delta^0 + x\Delta^1 + \frac{x(x-1)}{2!}\Delta^2 + \dots + \frac{x(x-1)\dots(x-r+1)}{r!}\Delta^r \quad (\text{VII})$$

when the given values of  $x$  are taken to be 0, 1, . . . ,  $n-1$ .

The condition that  $\xi_r$  is orthogonal to powers of  $x$  up to  $(r-1)$  can be expressed by the equation

$$S[(x+1)(x+2)\dots(x+q-1)\xi_r] = 0 \quad (\text{VIII})$$

where  $q$  can have any of the values  $1, 2, \dots, r$  and the summation extends over the integral values of  $x$  from 0 to  $n-1$ .

Substituting for  $\xi_r$  its value given by (VII), we have for the term in  $\Delta^p$ ,

$$S \left[ \frac{(x+q-1)(x+q-2) \dots (x-p+1)}{p!} \Delta^p \right].$$

We can write this in the difference form

$$S \left[ \frac{\Delta^p}{(\rho+q)p!} \left\{ (x+q)(x+q-1) \dots (x-p+1) - (x+q-1)(x+q-2) \dots (x-p) \right\} \right].$$

In this way the expression may be easily summed, and reduces to

$$\frac{(n+q-1)(n+q-2) \dots (n-p)}{p! (q+p)} \Delta^p.$$

It follows, therefore, from equation (VIII) that the conditions to be fulfilled are that for all integral values of  $q$  from 1 to  $r$

$$0 = \sum_{p=0}^r \frac{(n+q-1)!}{p! (n-p-1)!} \frac{\Delta^p}{q+p} \dots \dots \dots (1X)$$

$$= \frac{(n+q-1)!}{(n-1)!} \sum_{p=0}^r \frac{(n-1)!}{p! (n-p-1)!} \frac{\Delta^p}{q+p} \dots \dots \dots (X)$$

#### EVALUATION OF THE TERMINAL DIFFERENCES.

Now we can determine a form for  $\Delta^p$  which will satisfy the equation (X).

Consider the function

$$y = C(x+1)(x+2) \dots (x+r) \dots \dots \dots (XI)$$

This is a polynomial of degree  $r$  such that

$$y_p = C \frac{(\rho+r)!}{p!} \dots \dots \dots (XII)$$

Expressing  $y$  by means of the identity (familiar through Lagrange's use of it as an interpolation formula) in terms of the values  $y_0, y_1, \dots, y_r$  we have:

$$y = x(x-1) \dots (x-r) \sum_{p=0}^r \frac{y_p (-1)^{r-p}}{p! (r-p)! (x-p)} \dots \dots \dots (XIII)$$

Then when  $x = -q$  this gives

$$y_{-q} = (-1)^r q(q+1) \dots (q+r) \sum_{p=0}^r \frac{(-1)^{r-p}}{p! (r-p)!} \frac{y_p}{q+p} \dots \dots \dots (XIV)$$

and if

$$y_p = \frac{(n-1)! (r-p)!}{(n-p-1)!} (-1)^{r-p} \Delta^p \dots \dots \dots (XV)$$

then (XIV) becomes

$$y_{-q} = (-1)^r q(q+1) \dots (q+r) \sum_{p=0}^r \frac{(n-1)!}{p! (n-p-1)!} \frac{\Delta^p}{q+p} \quad (\text{XVI})$$

But it is clear from equation (XI) that  $y_{-q}$  vanishes for the values 1, 2, . . . ,  $r$  of  $q$ , and (XVI) therefore gives

$$0 = \sum_{p=0}^r \frac{(n-1)!}{p! (n-p-1)!} \frac{\Delta^p}{q+p}$$

for the values 1, 2, . . . ,  $r$  of  $q$ .

Hence the condition (X) is satisfied if we give to  $\Delta^p$  the value determined by equation (XV); that is, if we take

$$\Delta^p = \frac{(n-p-1)! (p+r)!}{(n-1)! (r-p)! p!} (-1)^{r-p} C \quad (\text{XVII})$$

The constant  $C$  is fixed by the convention that the coefficient of  $x^r$  in  $\xi_r$  is to be unity, so that the constant  $r$ th difference is  $r!$ . This makes

$$C = \frac{(r!)^2 (n-1)!}{(2r)! (n-r-1)!}, \quad (\text{XVIII})$$

and consequently

$$\Delta^p = \frac{(r!)^2 (p+r)! (n-p-1)!}{(2r)! p! (r-p)! (n-r-1)!} (-1)^{r-p} \quad (\text{XIX})$$

This value of  $\Delta^p$  substituted in equation (VII) gives one form for  $\xi_r$ ; it is

$$\frac{(-1)^r (r!)^2 (n-1)!}{(2r)! (n-r-1)!} + \sum_{p=1}^r \left\{ (-1)^{r-p} \frac{(r!)^2 (p+r)! (n-p-1)!}{(2r)! (p!)^2 (r-p)! (n-r-1)!} x(x-1) \dots (x-p+1) \right\} \quad (\text{A})$$

#### THE FORMULA FOR $S(\xi_r^2)$ .

Before proceeding to find a new general form for  $\xi_r$  from central differences let us establish the expression for  $S(\xi_r^2)$  from the terminal difference formula (XIX).

Because  $\xi_r$  is orthogonal to powers of  $x$  up to  $(r-1)$ , it follows that when we make  $q=r+1$ ,

$$S[(x+1) \dots (x+q-1)\xi_r] = S(\xi_r^2) \quad (\text{XX})$$

But  $S[(x+1) \dots (x+q-1)\xi_r]$  has already been given in equation (IX).

Substituting  $q=r+1$  in the expression for this sum we find

$$S(\xi_r^2) = \sum_{p=0}^r \frac{(n+r)!}{p! (n-p-1)!} \frac{\Delta^p}{p+r+1},$$

and substituting  $-x=q=(r+1)$  in the equation

$$C(x+1)(x+2) \dots (x+r) = x(x-1) \dots (x-r) \sum_{p=0}^r \frac{(n-1)!}{p! (n-p-1)!} \frac{\Delta^p}{x-p}$$



we deduce

$$r! C = \frac{(2r+1)!}{r!} \sum_{p=0}^r \frac{(n-1)!}{p! (n-p-1)!} \frac{\Delta^p}{p+r+1}.$$

It follows that

$$\sum_{p=0}^r \frac{(n+r)!}{p! (n-p-1)!} \frac{\Delta^p}{p+r+1} = \frac{(r!)^2 (n+r)!}{(2r+1)! (n-1)!} C$$

and substituting for C, we find

$$\begin{aligned} S(\xi_r^2) &= \frac{(r!)^4}{(2r)! (2r+1)!} \frac{(n+r)!}{(n-r-1)!} \\ &= \frac{(r!)^4}{(2r)! (2r+1)!} n(n^2-1)(n^2-4)(n^2-9) \dots (n^2-r^2). \end{aligned} \quad (\text{XXI})$$

### THE DIFFERENCE FORMULA.

Tchebycheff's difference formula follows immediately as a corollary to this. Instead of  $x$  we shall now use the new independent variate  $\xi_1$  where

$$\xi_1 = x - \frac{n-1}{2}.$$

That is to say, we shall consider  $\xi_r$  expressed as a function of  $\xi_1$  which is the deviation from the mean of the given series. The function  $\xi_r - \xi_1 \xi_{r-1}$  is a polynomial of degree  $r-2$  in  $\xi_1$  because the coefficient of  $\xi_1^r$  in  $\xi_r$  is unity, and the polynomials contain only alternate powers of  $\xi_1$ . Also it is orthogonal to powers of  $\xi_1$  up to  $r-3$ , since  $\xi_{r-1}$  is orthogonal up to  $(r-2)$ . It follows that  $\xi_r - \xi_1 \xi_{r-1}$  must be a multiple of  $\xi_{r-2}$ . If we write

$$\xi_r - \xi_1 \xi_{r-1} = \lambda \xi_{r-2},$$

then, multiplying by  $\xi_1^{r-2}$  and summing, we find

$$-S(\xi_1^{r-1} \xi_{r-1}) = \lambda S(\xi_1^{r-2} \xi_{r-2}),$$

where the summation extends over the values from  $-\frac{n-1}{2}$  to  $\frac{n-1}{2}$  of  $\xi_1$ .

This is equivalent to

$$\lambda = -\frac{S(\xi_{r-1}^2)}{S(\xi_{r-2}^2)}.$$

That is,

$$\lambda = -\frac{(r-1)^2 \{n^2 - (r-1)^2\}}{4(2r-1)(2r-3)};$$

and we deduce the relation

$$\xi_r = \xi_1 \xi_{r-1} - \frac{(r-1)^2 \{n^2 - (r-1)^2\}}{4(2r-1)(2r-3)} \cdot \xi_{r-2} \quad (\text{XXII})$$

which is the same as Tchebycheff's relation when here we put  $\frac{r!}{(2r)!} \xi_r$  instead of  $\xi_r$ .

GENERAL EXPRESSION FOR  $\xi_r$ .

By converting the terminal differences to central differences we can now give a general expression for the polynomial of degree  $r$ . According to the value given in equation (XIX) for  $\Delta^p$ , the  $(r-p)$ th terminal difference of  $\xi_r$  is

$$\Delta^{r-p} = (-1)^p \frac{(r!)^2 (2r-p)! (n-r+p-1)!}{p! (r-p)! (2r)! (n-r-1)!} \quad \text{(XXIII)}$$

We want to form the central differences at the mid-point of the series, that is corresponding to the value 0 of  $\xi_1$ . Let  $\delta^p \xi_r$  represent the  $p$ th central difference of  $\xi_r$ .

When  $n$  is odd, even central differences exist at the mid-point, and when  $n$  is odd and  $r$  even, the polynomial may be expressed in terms of these even central differences; equally when  $n$  is even and  $r$  odd, the polynomial may be expressed in terms of the odd central differences. When  $n$  and  $r$  are both even or both odd, the same formulæ may be used, though the differences which appear in them are really differences of the polynomial values at the successive mid-points between those actually used for the observations. In this way an  $r$ th difference comes always at the midpoint.

Thus the expression for the central difference  $\delta^{r-2s} \xi_r$  in terms of the terminal differences found above is

$$\begin{aligned} \Delta^{r-2s} &+ \frac{n-r+2s-1}{2 \cdot 1!} \Delta^{r-2s+1} + \frac{(n-r+2s-1)(n-r+2s-3)}{2^2 \cdot 2!} \Delta^{r-2s+2} \\ &+ \dots + \frac{(n-r+2s-1)(n-r+2s-3) \dots (n-r-2s+1)}{2^{2s} (2s)!} \Delta^r, \end{aligned}$$

and when we substitute in this the value given in (XXIII) for  $\Delta^{r-p}$  we find

$$\delta^{r-2s} \xi_r = (-1)^s \frac{r! (r-s-\frac{1}{2})!}{2^{2s} (r-\frac{1}{2})! s!} [n^2 - (r-1)^2][n^2 - (r-3)^2] \dots [n^2 - (r-2s+1)^2], \quad \text{(XXIV)}$$

where, for the sake of uniformity, the symbol  $p!$  is used instead of  $\Gamma(p+1)$  whether  $p$  be integral or fractional, since the properties of the two functions are identical.

Now  $\xi_r$  contains only alternate powers of  $\xi_1$ , and it follows that the adjacent differences  $\delta_{-\frac{1}{2}}^{r-2s-1} \xi_r$  and  $\delta_{\frac{1}{2}}^{r-2s-1} \xi_r$  are equal in magnitude but opposite in sign, and therefore we can express the function at once in terms of its central differences.

If  $r$  is even, the central differences that we have found are even differences, and in terms of these we get the following form for  $\xi_r$ :

$$\xi_r = \delta^0 \xi_r + \sum_{s=0}^{\frac{r-2}{2}} \delta^{r-2s} \xi_r \frac{\xi_1^2 (\xi_1^2 - 1^2) (\xi_1^2 - 2^2) \dots \left[ \xi_1^2 - \left( \frac{r-2-2s}{2} \right)^2 \right]}{(r-2s)!} \quad \text{(B}_1\text{)}$$

When  $r$  is odd, the central differences are odd, and for this case we have

$$\xi_r = \xi_1 \delta^1 \xi_r + \sum_{s=0}^{\frac{r-3}{2}} \delta^{r-2s} \xi_r \frac{\xi_1 (\xi_1^2 - \frac{1}{4}) (\xi_1^2 - \frac{9}{4}) \dots \left[ \xi_1^2 - \left( \frac{r-2-2s}{2} \right)^2 \right]}{(r-2s)!} \quad (B_2)$$

When the value of  $\delta^{r-2s} \xi_r$  is substituted from equation (XXIV), it is seen that the two results (B<sub>1</sub>) and (B<sub>2</sub>) can be included in the one general formula for the polynomial of degree  $r$ :

$$\frac{r!}{(r-\frac{1}{2})!} [\frac{1}{2}n]^r \xi_1 \sum_{q=0}^{\infty} \frac{(-)^q (r-q-\frac{1}{2})!}{(r-2q)! q! 2^{2q}} \frac{[\xi_1]^{r-2q-1}}{[\frac{1}{2}n]^{r-2q}}, \quad (C)$$

where the symbol  $[ \quad ]$  is defined by the equation

$$[x]^n = \left\{ x + \frac{1}{2}(n-1) \right\}! \left\{ x - \frac{1}{2}(n+1) \right\}!$$

and the series is summed for values of  $q$  upwards until  $2q$  is greater than  $r$ , when the denominator vanishes. The series therefore terminates in  $\frac{1}{2}(r+1)$  or  $\frac{1}{2}(r+2)$  terms.

(C) is thus the general expression in a series of central factorials for the polynomial of degree  $r$ . For small values of  $r$  it is readily expanded, giving the explicit expressions for the polynomials.

As far as  $\xi_{10}$  these are:—

$\xi_1$

$$\xi_1^2 - \frac{n^2 - 1}{12}$$

$$\xi_1^3 - \frac{3n^2 - 7}{20} \xi_1$$

$$\xi_1^4 - \frac{3n^2 - 13}{14} \xi_1^2 + \frac{3(n^2 - 1)(n^2 - 9)}{560}$$

$$\xi_1^5 - \frac{5(n^2 - 7)}{18} \xi_1^3 + \frac{15n^4 - 230n^2 + 407}{1008} \xi_1$$

$$\xi_1^6 - \frac{5(3n^2 - 31)}{44} \xi_1^4 + \frac{5n^4 - 110n^2 + 329}{176} \xi_1^2 - \frac{5(n^2 - 1)(n^2 - 9)(n^2 - 25)}{14784}$$

$$\xi_1^7 - \frac{7(3n^2 - 43)}{52} \xi_1^5 + \frac{7(15n^4 - 450n^2 + 2051)}{2288} \xi_1^3 - \frac{35n^6 - 1645n^4 + 17297n^2 - 27207}{27456} \xi_1$$

$$\begin{aligned} \xi_1^8 - \frac{7(n^2 - 19)}{15} \xi_1^6 + \frac{7(3n^4 - 118n^2 + 763)}{312} \xi_1^4 - \frac{105n^6 - 6405n^4 + 91679n^2 - 231491}{34320} \xi_1^2 \\ + \frac{7(n^2 - 1)(n^2 - 9)(n^2 - 25)(n^2 - 49)}{329472} \end{aligned}$$

# 320 Form of the Orthogonal Polynomials for Simple Series.

$$\begin{aligned} \xi_1^9 - \frac{3(3n^2 - 73)}{17} \xi_1^7 + \frac{21(3n^4 - 150n^2 + 1307)}{680} \xi_1^5 - \frac{21n^6 - 1617n^4 + 30387n^2 - 112951}{3536} \xi_1^3 \\ + \frac{3(105n^8 - 11060n^6 + 334054n^4 - 2973140n^2 + 4370361)}{3111680} \xi_1 \\ \xi_1^{10} - \frac{15(3n^2 - 91)}{76} \xi_1^8 + \frac{21(15n^4 - 930n^2 + 10507)}{2584} \xi_1^6 - \frac{5(21n^6 - 1995n^4 + 47775n^2 - 245737)}{10336} \xi_1^4 \\ + \frac{3(105n^8 - 13580n^6 + 514990n^4 - 6039260n^2 + 13782993)}{1074944} \xi_1^2 \\ - \frac{63(n^2 - 1)(n^2 - 9)(n^2 - 25)(n^2 - 49)(n^2 - 81)}{47297536}. \end{aligned}$$

In conclusion I wish to thank Dr R. A. Fisher for permitting me to use his results, and for the great amount of help which he has given me; also I am indebted to Dr J. Wishart for his advice and help.

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*Inverse Probability.* By R. A. FISHER, Sc.D., F.R.S., Gonville and Caius College; Statistical Dept., Rothamsted Experimental Station.

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I know only one case in mathematics of a doctrine which has been accepted and developed by the most eminent men of their time, and is now perhaps accepted by men now living, which at the same time has appeared to a succession of sound writers to be fundamentally false and devoid of foundation. Yet that is quite exactly the position in respect of inverse probability. Bayes, who seems to have first attempted to apply the notion of probability, not only to effects in relation to their causes but also to causes in relation to their effects, invented a theory, and evidently doubted its soundness, for he did not publish it during his life. It was posthumously published by Price, who seems to have felt no doubt of its soundness. It and its applications must have made great headway during the next 20 years, for Laplace takes for granted in a highly generalised form what Bayes tentatively wished to postulate in a special case.

Before going over the formal mathematical relationships in terms of which any discussion of the subject must take place, there are two preliminary points which require emphasis. First, it is not to be lightly supposed that men of the mental calibre of Laplace and Gauss, not to mention later writers who have accepted their views, could fall into error on a question of prime theoretical importance, without an uncommonly good reason. The underlying mental cause is not to be confused with the various secondary errors into which one is naturally led in deriving a formal justification of a false position, such as for example Laplace's introduction into his definition of probability of the unelucidated phrase "equally possible cases" which, since we must be taken to know what cases are equally possible before we know that they are equally probable, can only lead to the doctrine, known as the "doctrine of insufficient reason," that cases are equally probable (to us) unless we have reason to think the contrary, and so reduces all probability to a subjective judgment. The underlying mental cause is, I suggest, not to be found in these philosophical entanglements, but in the fact that we learn by experience that science has its inductive processes, so that it is naturally thought that such inductions, being uncertain, must be expressible in terms of probability. In fact, the argument runs somewhat as follows: a number of useful but uncertain judgments can be expressed with exactitude in terms of probability; our judgments respecting causes or hypotheses are uncertain, therefore our rational attitude towards them is expressible

in terms of probability. The assumption was almost a necessary one seeing that no other mathematical apparatus existed for dealing with uncertainties.

The second point is that the development of the subject has reduced the original question of the inverse argument in respect of probabilities to the position of one of a series of quite analogous questions; the hypothetical value, or parameter of the population under discussion, may be a probability, but it may equally be a correlation, or a regression, or, in genetical problems, a linkage value, or indeed any physical magnitude about which the observations may be expected to supply information. The introduction of quantitative variates, having continuous variation in place of simple frequencies as the observational basis, makes also a remarkable difference to the kind of inference which can be drawn.

It will be necessary to summarise some quite obvious properties of these continuous frequency distributions. The probability that a variate  $x$  should have a value in the range  $x \pm \frac{1}{2}dx$  is expressed as a function of  $x$  in the form

$$df = \phi(x) dx.$$

The function depends of course on the particular population from which the value of  $x$  is regarded as a random sample, and specifies the distribution in that population. If in the specification of the population one or more parameters,  $\theta_1, \theta_2, \theta_3, \dots$  are introduced, we have

$$df = \phi(x, \theta_1, \theta_2, \theta_3, \dots) dx,$$

where  $\phi$  now specifies only the form of the population, the values of its parameters being represented by  $\theta_1, \theta_2, \theta_3, \dots$ .

Knowing the distribution of the variate  $x$ , we also know the distribution of any function of  $x$ , for if

$$x = \chi(\xi)$$

we may substitute for  $x$  and obtain the distribution of  $\xi$  in the form

$$df = \phi\{\chi(\xi)\} \frac{d\chi}{d\xi} d\xi.$$

Obviously the form of the distribution has changed; thus, if we know the frequency distribution of the time in which a number of men run 100 yards, we may derive the distribution of their velocities, which will be a different distribution, obtained simply by transforming  $df$  as a differential element. In particular we must notice that the mean of the distribution is not invariant for such transformations, thus, if  $\bar{x}$  and  $\bar{\xi}$  are the means of their respective distributions, we shall not in general find that

$$\bar{x} = \chi(\bar{\xi}).$$

Similarly, the *mode*, that is, the point, if there is one, at which  $\phi$  has a maximum for variation of  $x$ , will not be invariant, for the equations

$$\frac{d^2 f}{dx^2} = 0, \quad \frac{d^2 f}{d\xi^2} = 0$$

will not normally be satisfied by corresponding values. The central measure which is invariant, at least if  $d\chi/d\xi$  is positive for all values, is the *median*, the value which divides the total frequency into two equal halves. For this point  $f = \frac{1}{2}$ , and the values of  $x$  and  $\xi$  will be necessarily in agreement. The same will be true of all other points defined by the value of  $f$ , so that we may have deciles, centiles, etc., dividing the frequency into 10 or 100 equal parts, and these will be invariant for any transformation for which  $d\chi/d\xi$  is always positive.

All the above applies with no essential change to the more general case in which we have several observable variates  $x, y, z, \dots$  in place of one.

The general statement of the inverse type of argument is as follows; we shall first cloak its fallacy under an hypothesis, and then examine it as an undisguised assumption.

Suppose that we know that the population from which our observations were drawn had itself been drawn at random from a super-population of known specification; that is, suppose that we have *a priori* knowledge that the probability that  $\theta_1, \theta_2, \theta_3, \dots$  shall lie in any defined infinitesimal range  $d\theta_1 d\theta_2 d\theta_3 \dots$  is given by

$$dF = \Psi(\theta_1, \theta_2, \theta_3, \dots) d\theta_1 d\theta_2 d\theta_3 \dots,$$

then the probability of the successive events (*a*) drawing from the super-population a population with parameters having the particular values  $\theta_1, \theta_2, \theta_3, \dots$  and (*b*) drawing from such a population the sample values  $x_1, \dots, x_n$ , will have a joint probability

$$\Psi(\theta_1, \theta_2, \theta_3, \dots) d\theta_1 d\theta_2 d\theta_3 \dots \times \prod_{p=1}^n \{\phi(x_p, \theta_1, \theta_2, \theta_3, \dots) dx_p\}.$$

If we integrate this over all possible values of  $\theta_1, \theta_2, \theta_3, \dots$  and divide the original expression by the integral we shall then have a perfectly definite value for the probability (in view of the observed sample and of our *a priori* knowledge) that  $\theta_1, \theta_2, \theta_3, \dots$  shall lie in any assigned limits.

This is not inverse probability strictly speaking, but a perfectly direct argument, which gives us the frequency distribution of the population parameters  $\theta$ , from which we may, if we like, calculate their means, modes, medians or whatever else might be of use.

The peculiar feature of the inverse argument proper is to say something equivalent to "We do not know the function  $\Psi$  specifying the super-population, but in view of our ignorance of the actual values of  $\theta$  we may take  $\Psi$  to be constant." Perhaps we might add that all values of  $\theta$  being equally possible their probabilities are by definition equal; but however we might disguise it, the choice of this particular *a priori* distribution for the  $\theta$ 's is just as arbitrary as any other could be. If we were, for example, to replace our  $\theta$ 's by an equal number of functions of them,  $\theta_1', \theta_2', \theta_3', \dots$  all objective statements could be translated from the one notation to the other, but the simple assumption  $\Psi(\theta_1, \theta_2, \theta_3, \dots) = \text{constant}$  may translate into a most complicated frequency function for

$$\theta_1', \theta_2', \theta_3', \dots$$

If, then, we follow writers like Boole, Venn, and Chrystal in rejecting the inverse argument as devoid of foundation and incapable even of consistent application, how are we to avoid the staggering falsity of saying that however extensive our knowledge of the values of  $x$  may be, yet we know nothing and can know nothing about the values of  $\theta$ ? Inverse probability has, I believe, survived so long in spite of its unsatisfactory basis, because its critics have until recent times put forward nothing to replace it as a rational theory of learning by experience.

The first point to be made belongs to the theory of statistical estimation; it has nothing to do with inverse probability, save for the historical accident that it was developed by Gauss in terms of that theory.

If we make the assumption that  $\Psi(\theta_1, \theta_2, \theta_3, \dots) = \text{constant}$ , and if then we ignore everything about the inverse probability distribution so obtained except its mode or point at which the ordinate is greatest, we have to maximise

$$\prod_{p=1}^n \{\phi(x_p, \theta_1, \theta_2, \theta_3, \dots)\}$$

for variations of  $\theta_1, \theta_2, \theta_3, \dots$ ; and the result of *this* process will be the same whether we use the parameters  $\theta_1, \theta_2, \theta_3, \dots$  or any functions of them,  $\theta_1', \theta_2', \theta_3', \dots$ . Two wholly arbitrary elements in this process have in fact cancelled each other out, the non-invariant process of taking the mode, and the arbitrary assumption that  $\Psi$  is constant. The choice of the mode is thinly disguised as that of "the most probable value," whereas had the inverse probability distribution any objective reality at all we should certainly, at least for a single parameter, have preferred to take the mean or the median value. In fact neither of these two processes has a logical justification, but each is necessary to eliminate the errors introduced by the other.



The process of maximising  $\Pi(\phi)$  or  $S(\log \phi)$  is a method of estimation known as the "method of maximum likelihood"; it has in fact no logical connection with inverse probability at all. The facts that it has been accidentally associated with inverse probability, and that when it is examined objectively in respect of the properties in random sampling of the estimates to which it gives rise, it has shown itself to be of supreme value, are perhaps the sole remaining reasons why that theory is still treated with respect. The function of the  $\theta$ 's maximised is not however a probability and does not obey the laws of probability; it involves no differential element  $d\theta_1 d\theta_2 d\theta_3 \dots$ ; it does none the less afford a rational basis for preferring some values of  $\theta$ , or combination of values of the  $\theta$ 's, to others. It is, just as much as a probability, a numerical measure of rational belief, and for that reason is called the *likelihood* of  $\theta_1, \theta_2, \theta_3, \dots$  having given values, to distinguish it from the probability that  $\theta_1, \theta_2, \theta_3, \dots$  lie within assigned limits, since in common speech both terms are loosely used to cover both types of logical situation.

If  $A$  and  $B$  are mutually exclusive possibilities the probability of " $A$  or  $B$ " is the sum of the probabilities of  $A$  and of  $B$ , but the likelihood of  $A$  or  $B$  means no more than "the stature of Jackson or Johnson"; you do not know what it is until you know which is meant. I stress this because in spite of the emphasis that I have always laid upon the difference between probability and likelihood there is still a tendency to treat likelihood as though it were a sort of probability.

The first result is thus that there are two different measures of rational belief appropriate to different cases. Knowing the population we can express our incomplete knowledge of, or expectation of, the sample in terms of probability; knowing the sample we can express our incomplete knowledge of the population in terms of likelihood. We can state the relative likelihood that an unknown correlation is  $+0.6$ , but not the probability that it lies in the range  $.595-.605$ .

There are, however, certain cases in which statements in terms of probability can be made with respect to the parameters of the population. One illustration may be given before considering in what ways its logical content differs from the corresponding statement of a probability inferred from known *a priori* probabilities. In many cases the random sampling distribution of a statistic,  $T$ , calculable directly from the observations, is expressible solely in terms of a single parameter, of which  $T$  is the estimate found by the method of maximum likelihood. If  $T$  is a statistic of continuous variation, and  $P$  the probability that  $T$  should be less than any specified value, we have then a relation of the form

$$P = F(T, \theta).$$

If now we give to  $P$  any particular value such as .95, we have a relationship between the statistic  $T$  and the parameter  $\theta$ , such that  $T$  is the 95 per cent. value corresponding to a given  $\theta$ , and this relationship implies the perfectly objective fact that in 5 per cent. of samples  $T$  will exceed the 95 per cent. value corresponding to the actual value of  $\theta$  in the population from which it is drawn. To any value of  $T$  there will moreover be usually a particular value of  $\theta$  to which it bears this relationship; we may call this the "fiducial 5 per cent. value of  $\theta$ " corresponding to a given  $T$ . If, as usually if not always happens,  $T$  increases with  $\theta$  for all possible values, we may express the relationship by saying that the true value of  $\theta$  will be less than the fiducial 5 per cent. value corresponding to the observed value of  $T$  in exactly 5 trials in 100. By constructing a table of corresponding values, we may know as soon as  $T$  is calculated what is the fiducial 5 per cent. value of  $\theta$ , and that the true value of  $\theta$  will be less than this value in just 5 per cent. of trials. This then is a definite probability statement about the unknown parameter  $\theta$ , which is true irrespective of any assumption as to its *a priori* distribution.

Fiducial 5 % $p$	95 % $r$	Fiducial 5 % $p$	95 % $r$	Fiducial 5 % $p$	95 % $r$
-.995055	-.968551	-.761594	+.145340	+.761594	+.989816
-.993963	-.961623	-.716298	+.270475	+.800499	+.991770
-.992632	-.953179	-.664037	+.388574	+.833655	+.993335
-.991007	-.942894	-.604368	+.496089	+.861723	+.994593
-.989027	-.930375	-.537050	+.590725	+.885352	+.995608
-.986614	-.915151	-.462117	+.671557	+.905148	+.996427
-.983675	-.896661	-.379949	+.738849	+.921669	+.997091
-.980096	-.874240	-.291313	+.793711	+.935409	+.997628
-.975743	-.847110	-.197375	+.837715	+.946806	+.998066
-.970452	-.814372	-.099668	+.872590	+.956237	+.998421
-.964028	-.775019	0	+.900000	+.964028	+.998711
-.956237	-.727916	+.099668	+.921432	+.970452	+.998646
-.946806	-.671918	+.197375	+.938146	+.975743	+.999139
-.935409	-.605881	+.291313	+.951174	+.980096	+.999296
-.921669	-.528824	+.379949	+.961338	+.983675	+.999424
-.905148	-.440127	+.462117	+.969286	+.986614	+.999529
-.885352	-.339761	+.537050	+.975519	+.989027	+.999615
-.861723	-.228562	+.604368	+.980424	+.991007	+.999685
-.833655	-.108446	+.664037	+.984298	+.992632	+.999742
-.800499	+.017528	+.716298	+.987371	+.993963	+.999789
-.761594	+.145340	+.761594	+.989816	+.995055	+.999827

For example, if  $r$  is a correlation derived from only four pairs of observations, and  $\rho$  is the correlation in the population from which the sample was drawn, the relation between  $\rho$  and the 95 per cent. value of  $r$  is given in the following table, which has been calculated, from the distribution formula I gave in 1915, by Miss F. E. Allan. From the table we can read off the 95 per cent.  $r$  for any given  $\rho$ , or equally the fiducial 5 per cent.  $\rho$  for any given  $r$ . Thus if a value  $r = .99$  were obtained from the sample, we should have a fiducial 5 per cent.  $\rho$  equal to about .765. The value of  $\rho$  can then only be less than .765 in the event that  $r$  has exceeded its 95 per cent. point, an event which is known to occur just once in 20 trials. In this sense  $\rho$  has a probability of just 1 in 20 of being less than .765. In the same way, of course, any other percentile in the fiducial distribution of  $\rho$  could be found or, generally, the fiducial distribution of a parameter  $\theta$  for a given statistic  $T$  may be expressed as

$$df = -\frac{\partial}{\partial \theta} F(T, \theta) d\theta,$$

while the distribution of the statistic for a given value of the parameter is

$$df = \frac{\partial}{\partial T} F(T, \theta) dT.$$

I imagine that this type of argument, which supplies definite information as to the probability of causes, has been overlooked by the earlier writers on probability, because it is only applicable to statistics of continuous distribution, and not to the cases in regard to which the abstract arguments of probability theory were generally developed, in which the objects of observation were classified and counted rather than measured, and in which therefore all statistics have discontinuous distributions. Now that a number of problems of distribution have been solved, for statistics having continuous distribution, arguments of this type force themselves on our attention; and I have recently received from the American statistician, Dr M. Ezekiel, graphs giving to a good approximation the fiducial 5 per cent. points of simple and multiple correlations for a wide range of cases. It is therefore important to realise exactly what such a probability statement, bearing a strong superficial resemblance to an inverse probability statement, really means. The fiducial frequency distribution will in general be different numerically from the inverse probability distribution obtained from any particular hypothesis as to *a priori* probability. Since such an hypothesis may be true, it is obvious that the two distributions must differ not only numerically, but in their logical meaning. It would be perfectly possible, for example, to find an *a priori*

frequency distribution for  $\rho$  such that the inverse probability that  $\rho$  is less than .765 when  $r = .99$  is not 5 but 10 in 100. In concrete terms of frequency this would mean that if we repeatedly selected a population at random, and from each population selected a sample of four pairs of observations, and rejected all cases in which the correlation as estimated from the sample ( $r$ ) was not exactly .99, then of the remaining cases 10 per cent. would have values of  $\rho$  less than .765. Whereas apart from any sampling for  $\rho$ , we know that if we take a number of samples of 4, from the same or from different populations, and for each calculate the fiducial 5 per cent. value for  $\rho$ , then in 5 per cent. of cases the true value of  $\rho$  will be less than the value we have found. There is thus no contradiction between the two statements. The fiducial probability is more general and, I think, more useful in practice, for in practice our samples will all give different values, and therefore both different fiducial distributions and different inverse probability distributions. Whereas, however, the fiducial values are expected to be different in every case, and our probability statements are relative to such variability, the inverse probability statement is absolute in form and really means something different for each different sample, unless the observed statistic actually happens to be exactly the same.

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*A percentile table of the relation between the true and the observed correlation coefficient from a sample of 4.* By Miss F. E. ALLAN, M.A. (Melbourne); Statistical Dept., Rothamsted Experimental Station, Harpenden, Herts. (Communicated by Dr R. A. FISHER.)

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The exact distribution of the simple correlation coefficient was established by Dr R. A. Fisher in 1915 (*Biometrika*, x, p. 507). If  $\rho$  is the true correlation in the population, and  $r$  the sample value, then the frequency element is given by

$$df = \frac{(1-\rho^2)^{\frac{n-1}{2}} (1-r^2)^{\frac{n-4}{2}}}{\pi (n-3)!} \left( \frac{\partial}{\sin \theta \partial \theta} \right)^{n-2} \frac{\theta}{\sin \theta} dr,$$

where  $\theta = \cos^{-1}(-\rho r)$ .

Dr Fisher has shown how this expression may be integrated in certain cases, giving the probability that a sample value of the correlation should be exceeded. When  $n$ , the number of pairs in the sample, is even, the problem is solved by a direct process of integration by parts, and can therefore be easily carried out for small values of  $n$ . The determination of the 95 per cent. values of  $r$ , that is the values which will exceed  $r$  in 95 per cent. of random trials, for given values of  $\rho$ , or correspondingly, the values of  $z$  for given values of  $\zeta$  according to the transformation

$$z = \tanh^{-1} r,$$

$$\zeta = \tanh^{-1} \rho,$$

is then easily made.

The distribution of  $z$  has been considered (*Metron*, 1921, 1, No. 4), and the advantage of using  $z$  in tests of significance depends on the approximate normality and constancy of the distribution. There is a small bias depending on the value of  $\rho$ , which decreases as the size of the sample is increased. For small samples, where it is practicable to evaluate the true 95 per cent. values of  $z$ , we can compare these true values with those obtained on the assumption of a normal distribution of  $z$ , correcting for bias, and in this way we can gain an indication of the nature and extent of the errors involved in making this assumption in tests of significance where larger values of  $n$  occur.

The following table gives the values of  $z$  for values of  $\zeta$  at intervals of 0.1 from -3.0 to +3.0, for the simple case  $n=4$ . The frequency element of the  $r$  distribution is proportional to

$$\frac{1}{\sin^3 \theta} (\theta - 3 \cot \theta + 3 \theta \cot^2 \theta) dr,$$

and the integral is

$$\frac{1}{\sin^2 \theta} (1 - \theta \cot \theta).$$

To find the 95 per cent. values of  $r$  corresponding to given values of  $\rho$  it was necessary therefore to solve for  $\theta$  the equation

$$\frac{1}{\sin^2 \theta} (1 - \theta \cot \theta) = P \frac{\pi \rho}{(1 - \rho^2)^{\frac{1}{2}}} + \frac{1}{1 - \rho^2} \left( 1 - \frac{\rho}{(1 - \rho^2)^{\frac{1}{2}}} \cos^{-1} \rho \right),$$

where  $P = 0.05$ . The values of  $r$  were then obtained from the relation

$$r = -\frac{1}{\rho} \cos \theta.$$

The values of  $\rho$  used were found from the relation  $\rho = \tanh \zeta$ , and a similar conversion gave the values of  $z$  from the calculated values of  $r$ .

The table may also be used for the 5 per cent. values by changing the signs of  $\zeta$  and  $z$ .

*95 per cent. values of the transformed correlation,  $z$ , for different values of the correlation  $\zeta$  in the population sampled. Samples of 4 pairs of observations.*

$\zeta$	$z$	$\zeta$	$z$	$\zeta$	$z$
-3.0	-4.6784	-1.0	-2.6375	1.0	-0.1464
-2.9	-4.5781	-0.9	-2.5293	1.1	-0.0175
-2.8	-4.4779	-0.8	-2.4196	1.2	+0.1089
-2.7	-4.3776	-0.7	-2.3084	1.3	0.2327
-2.6	-4.2773	-0.6	-2.1953	1.4	0.3538
-2.5	-4.1769	-0.5	-2.0804	1.5	0.4724
-2.4	-4.0764	-0.4	-1.9633	1.6	0.5885
-2.3	-3.9758	-0.3	-1.8440	1.7	0.7024
-2.2	-3.8751	-0.2	-1.7224	1.8	0.8142
-2.1	-3.7742	-0.1	-1.5984	1.9	0.9243
-2.0	-3.6730	0.0	-1.4722	2.0	1.0328
-1.9	-3.5717	0.1	-1.3438	2.1	1.1399
-1.8	-3.4701	0.2	-1.2135	2.2	1.2458
-1.7	-3.3681	0.3	-1.0814	2.3	1.3508
-1.6	-3.2658	0.4	-0.9479	2.4	1.4549
-1.5	-3.1629	0.5	-0.8136	2.5	1.5583
-1.4	-3.0594	0.6	-0.6788	2.6	1.6612
-1.3	-2.9553	0.7	-0.5441	2.7	1.7635
-1.2	-2.8504	0.8	-0.4101	2.8	1.8654
-1.1	-2.7445	0.9	-0.2774	2.9	1.9670
-1.0	-2.6375	1.0	-0.1464	3.0	+2.0683





*The Moments of the Distribution for Normal Samples of Measures of  
Departure from Normality.*

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1. *The Appropriate Symmetric Functions of the Observations.*

If  $x_1 \dots x_n$  are the values of a variate observed in a sample of  $n$ , from any population, we may evaluate a series of statistics ( $k$ ) such that the mean value of  $k_p$  will be the  $p$ th cumulative moment function of the sampled population; the first three of these are defined by the equations:

$$k_1 = \frac{1}{n} S(x),$$

$$k_2 = \frac{1}{n-1} S(x - k_1)^2,$$

$$k_3 = \frac{n}{(n-1)(n-2)} S(x - k_1)^3;$$

then it has been shown (Fisher, 1929)\* that the cumulative moment functions

\* R. A. Fisher, "Moments and Product Moments of Sampling Distributions," 'Proc. Lond. Math. Society,' Series 2, vol. 30, pp. 199-238 (1929).

of the simultaneous distribution, in samples, of  $k_1, k_2, k_3, \dots$ , may be obtained by the direct application of a very simple combination procedure.

The simplest measure of departure from normality will then be

$$\gamma = k_3 k_2^{-3/2},$$

a quantity which is evidently independent of the units of measurement, and in samples from a symmetrical distribution will have a distribution symmetrical about the value zero. In testing the evidence provided by a sample, of departure from normality, the distribution of this quantity in normal samples is required.

Hitherto the exact values of the moments of this distribution have been unknown, though a method of calculating the moments for large samples, in a series of any number of powers of  $n^{-1}$ , has been given. It will be shown that the distribution may be investigated by means of a recurrence relation, which yields the moments of the distribution and seems well adapted for the investigation of its other properties.

## 2. The Recurrence Relation for $\gamma$ .

For values of  $p$  from 1 to  $n - 1$ , let us define  $\xi_p$  by the relation

$$\xi_p = (x_p - k_1) + \frac{1}{n-1} (x_n - k_1),$$

or,

$$x - k_1 = \xi - \frac{1}{n-1} (x_n - k_1).$$

Then evidently

$$\sum_1^{n-1} (\xi_p) = 0$$

and

$$\sum_1^n (x_p - k_1)^2 = (x_n - k_1)^2 \left( 1 + \frac{1}{n-1} \right) + \sum_1^{n-1} (\xi_p^2)$$

while

$$\sum_1^n (x_p - k_1)^3 = (x_n - k_1)^3 \left( 1 - \frac{1}{(n-1)^2} \right) - \frac{3}{n-1} (x_p - k_1) \sum_1^{n-1} (\xi_p^2) + \sum_1^{n-1} (\xi_p^3),$$

so that, if

$$\frac{n}{n-1} (x_n - k_1)^2 = \cot^2 \theta \cdot \sum_1^{n-1} (\xi_p^2),$$

we may express the ratio

$$\gamma_n = \frac{n\sqrt{n-1}}{n-2} S (x - k_1)^3 \div S^{3/2} (x - k_1)^2$$

in terms of the ratio

$$\gamma_{n-1} = \frac{(n-1)\sqrt{n-2}}{n-3} S (\xi^3) \div S^{3/2} (\xi^2)$$

in the recurrence relation

$$\gamma_n = \frac{n(n-3)}{(n-1)^{1/2}(n-2)^{3/2}} \sin^3 \theta \cdot \gamma_{n-1} - \frac{3\sqrt{n}}{n-2} \cos \theta \sin^2 \theta + \sqrt{n} \cos^3 \theta, \quad (1)$$

where  $\gamma_{n-1}$  is the value of  $\gamma$  calculated from the sample values excluding  $x_n$ , and  $\gamma_n$  is the value calculated from the whole sample of  $n$  values.

The value of the recurrence relation in this form lies in the fact that the distribution of  $\theta$  is independent of that of  $\gamma_{n-1}$ , for whatever may be the values of  $\xi_1, \dots, \xi_{n-1} \div \sqrt{S}(\xi^2)$ , if  $\sigma$  be the standard error of the population sampled the distribution of

$$t = (x_n - k_1) \sqrt{n/n-1}$$

will be

$$\frac{1}{\sigma \sqrt{2\pi}} e^{-t^2/2\sigma^2} dt;$$

hence if

$$c = t/\sqrt{S},$$

where  $S$  stands for  $S(\xi^2)$ , since the distribution of  $S$  is known to be

$$df = \frac{1}{(2\sigma^2)^{\frac{1}{2}(n-2)} \frac{n-4}{2}!} S^{\frac{1}{2}(n-4)} e^{-S/2\sigma^2} dS,$$

the distribution of  $c$  will be given by

$$df = \frac{dc}{\sigma \sqrt{2\pi}} \cdot \frac{(2\sigma^2)^{-\frac{1}{2}(n-2)}}{\frac{n-4}{2}!} \int_0^\infty c^{-\frac{S}{2\sigma^2}(1+c^2)} S^{\frac{1}{2}(n-3)} dS = \frac{\frac{n-3}{2}!}{\frac{n-4}{2}!} \frac{dc}{\sqrt{\pi} (1+c^2)^{\frac{n-1}{2}}};$$

or, if  $c$  is  $\cot \theta$ , the distribution of  $\theta$  is

$$df = \frac{\frac{n-3}{2}!}{\frac{n-4}{2}! \sqrt{\pi}} \sin^{n-3} \theta d\theta. \quad (2)$$

independently of the value of  $\gamma_{n-1}$ , as indeed is obvious if the sample is considered geometrically.

### 3. *The Distribution for Samples of 3.*

The terminal values of  $\gamma_n$  are given by putting  $\theta = 0$  and  $\pi$ , when  $\gamma_n = \pm \sqrt{n}$  irrespective of the values of  $\gamma_{n-1}$  which is indeed indeterminate at these values. The recurrence relation enables us also by means of a single integration to obtain the distribution of  $\gamma_n$  from that of  $\gamma_{n-1}$ , or alternatively to obtain the moments of the distribution of  $\gamma_n$  in terms of those of the distribution of  $\gamma_{n-1}$ . To utilise the recurrence relation in these ways we shall need the distribution of  $\gamma$  for the smallest possible samples, *i.e.*, for  $n = 3$ .

When  $n = 3$ , we may represent the 3 deviations of the observations from the mean of the population by

$$x_1 = b + a \cos \phi, \quad x_2 = b + a \cos \left( \phi + \frac{2\pi}{3} \right) \quad x_3 = b + a \cos \left( \phi + \frac{4\pi}{3} \right),$$

then the mean of the sample is  $b$ , and the statistics  $k_2$  and  $k_3$  are given by

$$\begin{aligned} k_2 &= \frac{a^2}{2} \left\{ \cos^2 \phi + \cos^2 \left( \phi + \frac{2\pi}{3} \right) + \cos^2 \left( \phi + \frac{4\pi}{3} \right) \right\} \\ &= \frac{3}{4} a^2 \\ k_3 &= \frac{3a^3}{2} \left\{ \cos^3 \phi + \cos^3 \left( \phi + \frac{2\pi}{3} \right) + \cos^3 \left( \phi + \frac{4\pi}{3} \right) \right\}, \end{aligned}$$

but

$$\cos^3 \phi = \frac{1}{4} (\cos 3\phi + 3 \cos \phi),$$

hence

$$k_3 = \frac{9}{8} a^3 \cos 3\phi,$$

and

$$\gamma \equiv k_3 k_2^{-3/2} = \sqrt{3} \cos 3\phi.$$

For the sampling distribution of  $\phi$ , since

$$\frac{\partial (x_1, x_2, x_3)}{\partial (b, a, \phi)} = -\frac{3\sqrt{3}}{2} a,$$

and

$$x_1^2 + x_2^2 + x_3^2 = 3b^2 - \frac{3}{2} a^2,$$

we have

$$df = \frac{1}{(\sigma \sqrt{2\pi})^3} \cdot \frac{3\sqrt{3}}{2} \int_{-\infty}^{\infty} e^{-\frac{3b^2}{2\sigma^2}} db \int_0^{\infty} a e^{-\frac{3a^2}{4\sigma^2}} \cdot d\phi,$$

which on integration with respect to  $b$  yields

$$df = \frac{1}{(\sigma \sqrt{2\pi})^2} \int_0^{\infty} \frac{3a}{2} e^{-\frac{3a^2}{4\sigma^2}} da \cdot d\phi,$$

and on integration with respect to  $a$ , yields simply

$$df = \frac{1}{2\pi} d\phi.$$

Since, we have already found  $\gamma$  as a function of  $\phi$ , we have on substitution

$$df = \frac{d\gamma}{\pi \sqrt{3 - \gamma^2}} \quad (3)$$

as the distribution of  $\gamma$  for the case  $n = 3$ , since  $\gamma$  takes any particular value six times as  $\phi$  changes from 0 to  $2\pi$ .

The distribution is, of course, symmetrical, and has the following even moments

$$\begin{aligned} \mu_2 &= 3/2 \\ \mu_4 &= 27/8 \\ \mu_6 &= 135/16 \\ &\dots\dots\dots \\ \mu_{2s} &= \frac{(s - \frac{1}{2})!}{s! \sqrt{\pi}} \cdot 3^s. \end{aligned}$$

#### 4. *The Moments of $\gamma$ in General.*

The exact distribution for  $n > 3$  seems not to be expressible simply in terms of known functions. For the moments about the mean (zero) of the distribution we may proceed as follows: let  $v_n$  be the variance of the distribution of  $\gamma_n$ , then squaring both sides of equation (1) and averaging over all possible values we find

$$\begin{aligned} v_n = \frac{n^2 (n-3)^2}{(n-1)(n-2)^3} \sin^6 \theta \cdot v_{n-1} + n \cos^6 \theta - \frac{6n}{n-2} \cos^4 \theta \sin^2 \theta \\ + \frac{9n}{(n-2)^2} \cos^2 \theta \sin^4 \theta; \end{aligned}$$

since equation (2) gives the distribution of  $\theta$  we may now average over all values of  $\theta$ , by multiplying by the right-hand side of this equation and integrating with respect to  $\theta$  from 0 to  $2\pi$ , we then have

$$\begin{aligned} v_n &= \frac{n^2 (n-3)^2}{(n-1)(n-2)^3} \cdot \frac{(n-2)n(n+2)}{(n-1)(n+1)(n+3)} \cdot v_{n-1} \\ &\quad + \frac{1}{(n-1)(n+1)(n+3)} \left\{ n \cdot 1 \cdot 3 \cdot 5 - \frac{6n}{n-2} \cdot 1 \cdot 3 \right. \\ &\quad \left. + \frac{9n}{(n-2)^2} (n-2)n \cdot 1 \right\} \\ &= \frac{n^3 (n+2)(n-3)^2}{(n-2)^2 (n-1)^2 (n+1)(n+3)} v_{n-1} + \frac{6n}{(n-2)(n-1)(n+3)}. \end{aligned}$$

The variance for any particular value of  $n$  may now be found by direct substitution; alternatively we may note that if

$$w_n = \frac{(n-2)^2 (n+1) (n+3)}{n^2} v_n,$$

then

$$w_{n-1} = \frac{(n-3)^2 n (n+2)}{(n-1)^2} v_{n-1},$$

and the recurrence relation is reduced to

$$w_n - w_{n-1} = \frac{6(n-2)(n+1)}{n(n-1)} = 6 + 12\left(\frac{1}{n} - \frac{1}{n-1}\right);$$

whence

$$w_n = C + 6n + 12/n,$$

where  $C$  is a constant to be determined from

$$w_3 = 4;$$

whence

$$w_n = 6\left(n - 3 + \frac{2}{n}\right) = \frac{6(n-1)(n-2)}{n} \frac{1}{2},$$

and

$$v_n = \frac{6n(n-1)}{(n-2)(n+1)(n+3)},$$

the general formula for the variance of  $\gamma_n$ .

The same process applied to the mean fourth power will give a recurrence formula involving the variance, for which the value found can now be substituted; in this way the mean values of all even powers may be evaluated in succession. Writing  $v'$  for the fourth moment, we have the equation

$$\begin{aligned} v_n' &= \frac{n^4 (n-3)^4}{(n-1)^2 (n-2)^6} \cdot \frac{(n-2)n(n+2)(n+4)(n+6)(n+8)}{(n-1)(n+1)(n+3)(n+5)(n+7)(n+9)} v_{n-1}' \\ &+ \frac{6n^3 (n-3)^2}{(n-1)(n-2)^3} \cdot \frac{6(n^2 - n + 70)n(n+2)}{(n-2)(n-1)(n+1)(n+3)(n+5)(n+7)(n+9)} v_{n-1} \\ &+ \frac{108n^3 (31n^3 - 144n^2 + 183n + 70)}{(n-2)^3 (n-1)(n+1)(n+3)(n+5)(n+7)(n+9)}, \end{aligned}$$

or, substituting for  $v_{n-1}$ ,

$$\begin{aligned} v_n' &= \frac{n^5 (n-3)^4 (n+2)(n+4)(n+6)(n+8)}{(n-2)^5 (n-1)^3 (n+1)(n+3)(n+5)(n+7)(n+9)} v_{n-1}' \\ &+ \frac{108n^3 (2n^4 + 23n^3 + 2n^2 - 237n + 70)}{(n-2)^3 (n-1)(n+1)(n+3)(n+5)(n+7)(n+9)}; \end{aligned}$$

but if

$$w_n' = \frac{(n-2)^4 (n+1)(n+3)(n+5)(n+7)(n+9)}{n^4 (n-1)} v_n',$$

then

$$\begin{aligned} w_n' - w_{n+1}' &= \frac{108 (n-2)}{n^2 (n-1)^2} (2n^4 + 23n^3 + 2n^2 - 237n + 70) \\ &= 108 \left\{ 2n + 23 - \frac{264}{n(n-1)} + 140 \frac{2n-1}{n^2 (n-1)^2} \right\}, \end{aligned}$$

so that

$$w_n' = 108 \left( n^2 + 24n + C + \frac{264}{n} - \frac{140}{n^2} \right),$$

where C is to be determined from

$$w_3' = 480$$

so that

$$C = -149,$$

$$w_n' = \frac{108 (n-1)(n-2)}{n^2} (n^2 + 27n - 70),$$

and the fourth moment of  $\gamma$  is given by

$$\mu_4(\gamma) = v_n' = \frac{108n^2 (n-1)^2 (n^2 + 27n - 70)}{(n-2)^3 (n+1)(n+3)(n+5)(n+7)(n+9)}$$

Similarly the sixth moment is found to be

$$\mu_6(\gamma) = \frac{3240n^3 (n-1)^3 (n^4 + 84n^3 + 2695n^2 - 15168n + 20020)}{(n-2)^5 (n+1)(n+3) \dots (n+15)},$$

and the same method may be applied to determine the higher moments.

From the moments the cumulative moment functions may be determined by the invariable relationships, which for symmetrical distributions become

$$\kappa_2 = \mu_2$$

$$\kappa_4 = \mu_4 - 3\mu_2^2$$

$$\kappa_6 = \mu_6 - 15\mu_2\mu_4 + 30\mu_2^3,$$

which give us the values

$$\kappa_2 = \frac{6n(n-1)}{(n-2)(n+1)(n+3)},$$

$$\kappa_4 = \frac{1296n^2 (n-1)^2 (n-7)(n^2 + 2n - 5)}{(n-2)^3 (n+1)^2 (n+3)^2 (n+5)(n+7)(n+9)},$$

$$\kappa_6 = \frac{466560n^3 (n-1)^3 (7n^6 - 88n^5 - 286n^4 + 3284n^3 + 1667n^2 - 22108n + 20020)}{(n-2)^5 (n+1)^3 (n+3)^3 (n+5)(n+7)(n+9)(n+11)(n+13)(n+15)},$$

from which we may derive the ratios,

$$\frac{1}{4!} \kappa_4 \kappa_2^{-2} = \frac{3(n-7)(n^2+2n-5)}{2(n-2)(n+5)(n+7)(n+9)},$$

$$\frac{1}{6!} \kappa_6 \kappa_2^{-3} = \frac{3(7n^6 - 88n^5 - 286n^4 + 3284n^3 + 1667n^2 - 22108n + 20020)}{(n-2)^2(n+5)(n+7)(n+9)(n+11)(n+13)(n+15)},$$

which determine the rate of approach of the distribution of  $\gamma$  to normality as the sample number  $n$  is increased. It will be noticed that  $\kappa_4$  changes from a negative to a positive sign at  $n = 7$ , and that the corresponding ratio rises to its greatest value about 0.024 at  $n = 22$ , while the corresponding ratio for  $\kappa_6$  starting from positive values has a negative maximum about  $-0.0016$  at  $n = 8$ , is positive again at  $n = 13$ , and reaches a positive maximum about  $+0.0027$  at  $n = 32$ . Using the reciprocal of  $n$  as abscissa the course of these two ratios is shown in figs. 1 and 2.

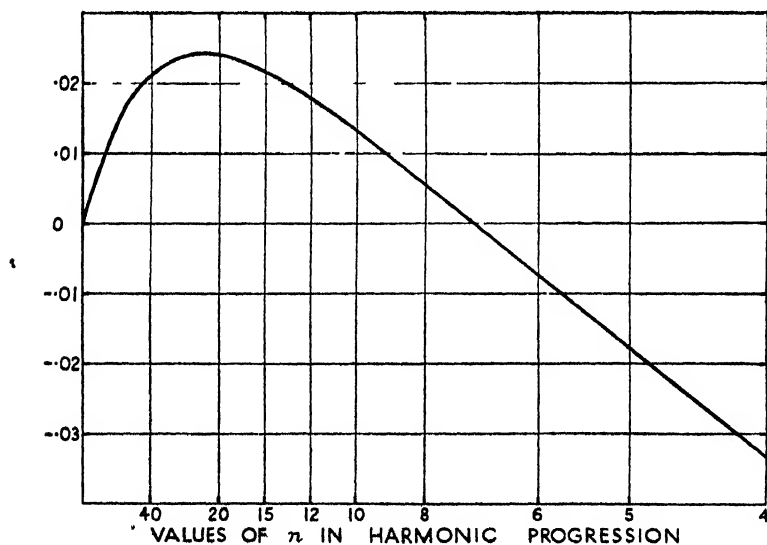


FIG. 1.—Graph of the Ratio  $\kappa_4 \kappa_2^{-2} / 4!$  of the distribution of  $\gamma = k_3 k_2^{-3/2}$ .



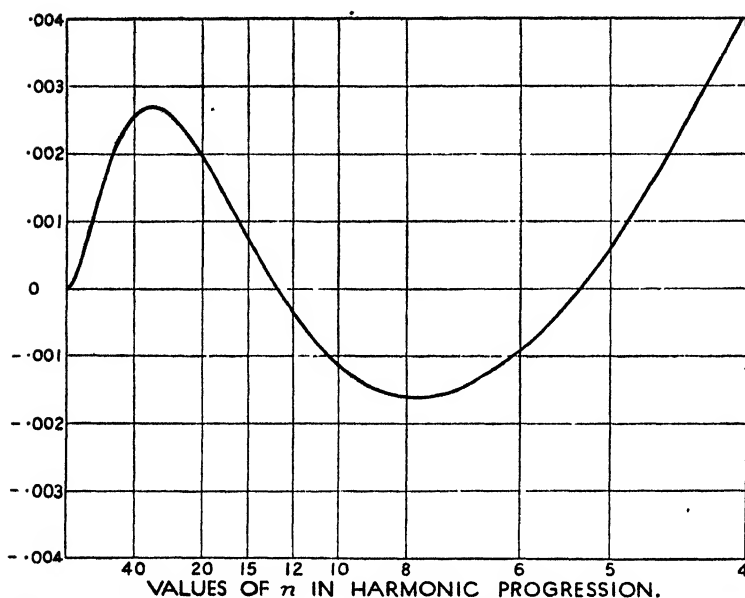


FIG. 2.—Graph of the Ratio  $\kappa_6\kappa_2^{-3}/6!$  of the distribution of  $\gamma = k_3k_2^{-3/2}$ .

### 5. The Moments of the Simultaneous Distribution of Different Measures of Departure from Normality.

It is obvious that the method of approach adopted in the foregoing sections is applicable to the determination of the moments of the distributions of, or more generally of the simultaneous distribution of, all measures of departure from normality such as

$$\gamma = k_3k_2^{-3/2}$$

$$\delta = k_4k_2^{-2}$$

$$\epsilon = k_5k_2^{-5/2}$$

and so on.

For  $\delta$  and  $\epsilon$  we find the recurrence relations comparable with that already found for  $\gamma$ , namely

$$\gamma_n = n^{1/2} c^3 - \frac{3n^{1/2}}{n-2} c s^2 + \frac{n(n-3)}{(n-2)^{3/2}(n-1)^{1/2}} s^3 \cdot \gamma_{n-1},$$

$$\begin{aligned} \delta_n = n c^4 - \frac{6n}{n-2} c^2 s^2 + \frac{3}{n-2} s^4 - \frac{4n^{1/2}(n+1)}{(n-2)^{3/2}(n-1)^{1/2}} c s^3 \gamma_{n-1} \\ + \frac{(n+1)(n-4)}{(n-2)^2} s^4 \cdot \delta_{n-1}, \end{aligned}$$

$$\begin{aligned} \epsilon_n = & n^{3/2} c^5 \frac{10n^{3/2}}{n-2} c^3 s^2 + \frac{15n^{1/2}}{n-2} c s^4 \frac{10n(n+1)}{(n-2)^{3/2}(n-1)^{1/2}} \left( c^2 s^3 - \frac{s^5}{n+4} \right) \gamma_{n-1} \\ & - \frac{5n^{1/2}(n+5)}{(n-2)^2} c s^4 \delta_{n-1} + \frac{n^2(n^2-25)s^5}{(n-2)^{5/2}(n-1)^{1/2}(n+4)} \epsilon_{n-1}, \end{aligned}$$

and by a mere repetition of the algebraic processes employed above, we may obtain a recurrence relation for the mean value of any expression of the form

$$\gamma^a \delta^b \epsilon^c, \dots,$$

from which the mean value in question may be derived.

If, in accordance with the notation employed for the designation of the moments of the set of statistics  $k_2, k_3, k_4, \dots$ , we represent such a mean product by

$$\mu(\dots 5^c 4^b 3^a 2^{-r}),$$

where

$$2r = 3a + 4b + 5c + \dots,$$

so that  $r$  is always an integer save for the odd moments which necessarily vanish, we may list the following formulæ:

$$\mu(3^2 2^{-3}) = \frac{6n(n-1)}{(n-2)(n+1)(n+3)},$$

$$\mu(4^2 2^{-4}) = \frac{24n(n-1)^2}{(n-3)(n-2)(n+3)(n+5)},$$

$$\mu(4^3 2^{-5}) = \frac{216n^2(n-1)^2}{(n-2)^2(n+1)(n+3)(n+5)(n+7)},$$

$$\mu(5^2 2^{-5}) = \frac{120n^2(n+5)(n-1)^3}{(n-4)(n-3)(n-2)(n+1)(n+3)(n+5)(n+7)},$$

$$\mu(3^4 2^{-6}) = \frac{108n^2(n-1)^2(n^2+27n-70)}{(n-2)^3(n+1)(n+3)(n+5)(n+7)(n+9)},$$

$$\mu(4^3 2^{-6}) = \frac{1728n(n-1)^3(n^2-5n+2)}{(n-3)^2(n-2)^2(n+3)(n+5)(n+7)(n+9)},$$

$$\mu(3^6 2^{-9}) = \frac{3240n^3(n-1)^3(n^4+84n^3+2695n^2-15168n+20020)}{(n-2)^5(n+1)(n+3)(n+5)(n+7)(n+9)(n+11)(n+13)(n+15)}.$$

A comparison of these formulæ with those already given (Fisher, 1929), for

the cumulative moment functions of  $k_2, k_3, k_4$ , which in every case but  $\mu(3^4)$  and  $\mu(3^6)$  are also the moments of the distribution, shows that

$$\begin{aligned}\mu(3^2) &= \frac{6n}{(n-1)(n-2)} \kappa_2^3, \\ \mu(4^2) &= \frac{24n(n+1)}{(n-1)(n-2)(n-3)} \kappa_2^4, \\ \mu(4^3) &= \frac{216n^2}{(n-1)^2(n-2)^2} \kappa_2^5, \\ \mu(5^2) &= \frac{120n^2(n+5)}{(n-1)(n-2)(n-3)(n-4)} \kappa_2^5, \\ \mu(4^3) &= \frac{1728n(n+1)(n^2-5n+2)}{(n-1)^2(n-2)^2(n-3)^2} \kappa_2^6.\end{aligned}$$

Moreover

$$\begin{aligned}\mu(3^4) &= \kappa(3^4) + 3\kappa^2(3^2) \\ &= \left\{ \frac{648n^2(5n-12)}{(n-1)^3(n-2)^3} + \frac{108n^2}{(n-1)^2(n-2)^2} \right\} \kappa_2^6 \\ &= \frac{108n^2(n^2+27n-70)}{(n-1)^3(n-2)^3} \kappa_2^6,\end{aligned}$$

and

$$\begin{aligned}\mu(3^6) &= \kappa(3^6) + 15\kappa(3^4)\kappa(3^2) + 15\kappa^3(3^2) \\ &= 3240 \kappa_2^9 \left\{ \frac{144(22n^2-111n+142)n^3}{(n-1)^5(n-2)^5} + \frac{18(5n-12)n^3}{(n-1)^4(n-2)^4} \right. \\ &\quad \left. + \frac{n^3}{(n-1)^3(n-2)^3} \right\} \\ &= \frac{3240n^3(n^4+84n^3+2695n^2-15168n+20020)}{(n-1)^5(n-2)^5} \kappa_2^9.\end{aligned}$$

In every case, therefore, the moment of the distribution of  $\gamma, \delta, \epsilon, \dots$ , is derivable by multiplying by

$$\frac{(n-1)^r}{(n-1)(n+1)\dots(n+2r-3)} \kappa_2^r$$

the corresponding moment of the distribution of  $k_3, k_4, k_5, \dots$ . Since many of the latter moments may be found relatively expeditiously by means of the combinatorial procedure, this will be the quicker method for the more complex product moments. For moments of high degree, however, such as  $(3^6)$  and  $(3^{10})$  it does not seem easy to enumerate with certainty all the combinatorial

patterns, and the recurrence method, though necessarily heavy, supplies a valuable check.

An analytical proof of this relationship, or at least analytical grounds for accepting it as general, may be found by the method of transforming the characteristic function previously employed in demonstrating the rules of the combinatorial method. If

$$M(t_1, t_2, \dots)$$

is the characteristic function of the simultaneous distribution of the variates  $x_1, x_2, \dots$ , and if

$$M'(\tau_1, \tau_2, \dots)$$

is that of variates  $\xi_1, \xi_2, \dots$ , defined in terms of  $x_1, x_2, \dots$ , by the relations

$$\xi_1 = f_1(x_1, x_2, \dots),$$

$$\xi_2 = f_2(x_1, x_2, \dots),$$

then

$$M'(\tau_1, \tau_2, \dots) = e^{\tau_1 f_1 + \tau_2 f_2 + \dots} M(t_1, t_2, \dots)$$

at  $t_1 = 0, t_2 = 0, \dots$ , where  $f_p$  in the index stands for

$$f_p \left( \frac{d}{dt_1}, \frac{d}{dt_2}, \dots \right).$$

To apply this theorem to the present case we utilise the fact that in sampling from the normal distribution  $k_2$  is distributed independently of  $\gamma, \delta, \epsilon, \dots$ , in the known distribution

$$df = \frac{1}{\frac{1}{2}(n-3)} \cdot \left( \frac{n-1}{2\kappa_2} \right)^{\frac{1}{2}(n-1)} k_2^{\frac{1}{2}(n-3)} e^{-\frac{1}{2}(n-1)k_2/\kappa_2} dk_2,$$

of which the characteristic function,

$$\int e^{it_2 k_2} df,$$

is

$$\left( 1 - \frac{2\kappa_2 t_2}{n-1} \right)^{-\frac{1}{2}(n-1)}.$$

Hence the general characteristic function of the simultaneous distribution of  $k_2, \gamma, \delta, \dots$ , is of the form

$$\left( 1 - \frac{2\kappa_2 t_2}{n-1} \right)^{-\frac{1}{2}(n-1)} M(t_3, t_4, \dots),$$

where  $M$  is the sum of terms such as

$$\mu \left( \dots 5^c 4^b 3^a 2^r \right) \frac{t_3^a}{a!} \frac{t_4^b}{b!} \frac{t_5^c}{c!} \dots,$$

and from this expression we must be able to derive the function

$$M'(\tau_3, \tau_4, \dots) = \Sigma \mu(\dots 5^c 4^b 3^a) \frac{\tau_3^a}{a!} \frac{\tau_4^b}{b!} \frac{\tau_5^c}{c!}$$

by the action of the operator

$$e^{\tau_3 D_1 D_1^{3/2} + \tau_4 D_1 D_1^2 + \tau_5 D_1 D_1^{5/2} + \dots}$$

where  $D_1$  stands for  $d/dt_1$ .

It appears, therefore, without discussing what meaning should be attached to the fractional indices, which find in fact only zero terms on which to operate, that

$$\mu(\dots 5^c 4^b 3^a) = \mu(\dots 5^c 4^b 3^a 2^{-r}) \cdot \frac{d^r}{dt_2^r} \left(1 - \frac{2\kappa_2 t_2}{n-1}\right)^{-\frac{1}{2}(n-1)}$$

at  $t_2 = 0$ , or that

$$\mu(\dots 5^c 4^b 3^a) = \mu(\dots 5^c 4^b 3^a 2^{-r}) \cdot \frac{(n-1) \dots (n+2r-3)}{(n-1)^r} \kappa_2^r,$$

which is the relationship required.

### *Summary.*

Two methods are given for discussing the distribution of the ratios of the symmetric functions  $k_3, k_4, \dots$ , obtained from samples from a normal distribution to the powers of  $k_2$  of the same degree.

The first method consists in the development of recurrence relations expressing the ratios from a sample of  $n$  in terms of the corresponding ratios from a sample of  $n-1$  observations, and of a parameter distributed independently in a known distribution. Theoretically all properties of the general distribution could be obtained from these relations in conjunction with a study of samples of 3, 4, 5 ... observations.

The relations are used to derive the exact values of the first three even moments of the simplest ratio  $\gamma$ , and of the simpler non-vanishing moments of the simultaneous distribution of all the ratios. It is observed that these moments are very simply related to the corresponding moments of the distribution of  $k_3, k_4, \dots$ , given in a previous paper.

The second method is an application of the method of symbolical operators developed by the author, which confirms the generality of the relationship found. The moments of the one distribution may thus be inferred directly from that of the other for which the combinatorial procedure is available.



# THE DERIVATION OF THE PATTERN FORMULAE OF TWO-WAY PARTITIONS FROM THOSE OF SIMPLER PATTERNS

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## 1. *Introductory.*

If the moment generating function of a variable be defined in terms of the frequency element  $df(x)$  of a variate  $x$  in the form

$$M = \int e^{tx} df(x),$$

then, when  $M$  can be expanded in a series of powers of  $t$ , the cumulative function  $K$ , defined by

$$K = \log M,$$

can also be expanded in the form

$$K = \sum_r \frac{\kappa_r t^r}{r!}.$$

The coefficients  $\kappa_r$  of this expansion have by different writers been termed semi-invariants and cumulative moment functions. Since both terms are lengthy, and the first somewhat misleading, we propose in what follows to refer to them as the *cumulants* of the distribution.

In a recent paper\* it was shown that, if symmetric functions  $k_r$ , of the  $r$ -th degree, are calculated from the observations of a sample, in such a manner that the mean value of  $k_r$  in all possible samples is equal to the  $r$ -th cumulant ( $\kappa_r$ ) of the infinite population from which the sample has been taken, then the cumulants of the distribution of any  $k$ , and of the simultaneous distribution of all such statistics, which are necessarily expressible in terms of the cumulants of the population, may be readily and simply determined by combinatorial methods. The essence of the method is that the coefficient of any single term in the required

\* R. A. Fisher, *Proc. London Math. Soc.* (2), 30 (1929), 199–238.

formulae is a composite of contributions from one or more two-way partitions, of which one marginal partition represents the formula, while the other specifies the particular term; and for each such partition the numerical coefficient is derived as the number of ways of setting up the partition, while the coefficient in  $n$ , the size of the sample, depends on the pattern of the two-way partition, *i.e.* on the number of rows and columns, and the number and distribution of zero entries. A useful list of such pattern formulae has already been given (*loc. cit.*).

The listing of the great number of patterns possible for two-way partitions of larger numbers would, however, be impracticable; the present paper will show how the pattern formulae for such cases may be readily derived from those already listed. Patterns with a large number of rows have necessarily, for formulae of given degree, relatively few entries in each row. The extreme case is that for the terms involving only the variance of the sampled population, in which each row has only two entries; these patterns are of particular interest, since only these occur in the distribution of moment statistics derived from the normal distribution. It will be seen that the method of deriving the pattern function by the addition of a new column is particularly simple in these cases. It should be noted that the pattern functions are the same for multivariate as for univariate problems.

## 2. The general method of evaluating a pattern.

The general procedure for determining the function of  $n$  associated with any specified pattern is to consider all the possible ways in which the rows can be separated into separate groups, or separates. Thus with two rows we have only two possible separations, the rows being either amalgamated as in the marginal total, or kept separate; these two separations correspond to the partitions (2) and (1<sup>2</sup>) respectively. With three rows we have one separation corresponding to the partition (3), three corresponding to the partition (21), and one corresponding to the partition (1<sup>3</sup>). In general the number of separations of  $r$  rows corresponding to the partition  $(p_1^{r_1} p_2^{r_2} \dots)$  is

$$\frac{r!}{\pi_1! (p_1!)^{r_1} \cdot \pi_2! (p_2!)^{r_2} \dots},$$

and the total number of separations of  $r$  rows into  $s$  separates is

$$\frac{1}{(s-1)!} \Delta^{s-1} (1^{r-1}),$$

the leading  $(s-1)$ -th divided difference of the series of the  $(r-1)$ -th



powers of the positive integers. Representative numbers are given in Table I.

TABLE I.  
Number of separations of  $r$  rows into  $s$  separates.

Number of rows	Number of separates									Total
	1	2	3	4	5	6	7	8	9	
2	1	1								2
3	1	3	1							5
4	1	7	6	1						15
5	1	15	25	10	1					52
6	1	31	90	65	15	1				203
7	1	63	301	350	140	21	1			877
8	1	127	966	1701	1050	266	28	1		4140
9	1	255	3025	7770	6951	2646	462	36	1	21147

The total number of separations, being [Whitworth, *Choice and Chance* (1886), 95] the coefficient of  $x^r/r!$  in the expansion of

$$e^{x-1},$$

increases rapidly, and although, usually, large groups of separations making similar contributions may be treated together, it is evidently desirable to shorten the method for cases of more than six or eight rows.

The contribution of each separation to the function required is the product of the factor  $n(n-1)\dots(n-s+1)$  with factors for the several columns; these factors are

$$\frac{1}{n}, \quad \frac{-1}{n(n-1)}, \quad \frac{2!}{n(n-1)(n-2)}, \quad \dots,$$

according as the column is represented in 1, 2, 3, ... separates. This process has been already sufficiently exemplified (R. A. Fisher, *loc. cit.*). We shall now show how the pattern-function for a pattern containing a column with only two entries may be obtained from the pattern-functions of simpler patterns, and then consider the parallel procedure to be used when no column contains less than three or more entries.

### 3. Expansion of a pattern function in terms of the functions of simpler patterns.

Consider the pattern

```

. x x x
x . x x
x x . .
x x . .

```

of which the right-hand column contains only two entries. The fifteen separations which are possible with four rows may be divided into two classes, in one of which ( $\alpha$ ) the two rows represented in the fourth column lie in the same separate, while in the second class ( $\beta$ ) they lie in different separates. In the first class the fourth column contributes the factor  $n^{-1}$ , in the second class the factor is  $-1/n(n-1)$ .

Now in separations of the first class the cofactors of  $n^{-1}$  will be the contributions of all its possible separations to the pattern-function of the pattern

$$\begin{array}{c} \times \times \times \\ \times \times . \\ \times \times . \end{array}$$

in which the fourth column is omitted, and the first two rows of the original pattern are amalgamated. In general we may designate the function of this reduced pattern by  $A$ . If now we represent the function of the pattern

$$\begin{array}{c} . \times \times \\ \times . \times \\ \times \times . \\ \times \times . \end{array}$$

in which the rows have not been amalgamated, by  $B$ , it appears that the cofactor of  $-1/n(n-1)$  in the pattern-function to be evaluated will consist of all the contributions to  $B$  which do not occur in  $A$ , and the required function is reduced to the form

$$\frac{A}{n} - \frac{B-A}{n(n-1)} \equiv \frac{A}{n-1} - \frac{B}{n(n-1)},$$

which is a general formula for the function of any pattern having a column with only two entries.

In the particular case considered we may at once substitute

$$A = \frac{1}{(n-1)(n-2)}, \quad B = \frac{n}{(n-1)^2(n-2)},$$

and obtain 
$$\frac{1}{(n-1)^2(n-2)} \left(1 - \frac{1}{n-1}\right) \equiv \frac{1}{(n-1)^3},$$

in accordance with the value given in Fisher's list of useful patterns.

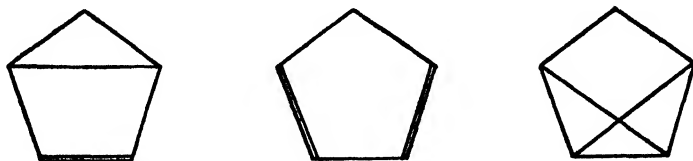
Alternatively, of course,  $B$  could have been reduced in turn to functions of two column patterns.

Whenever one or both rows represented in the column to be removed contain only two entries, the pattern-function  $B$  vanishes, and we are left simply with

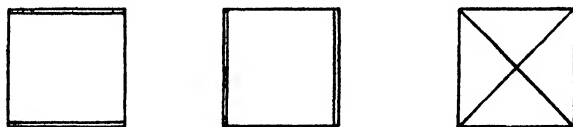
$$A/(n-1);$$

this will always be the case with the pattern of terms involving only variances and covariances, which are the only terms which appear in sampling from normal populations.

For these normal cases use may be made of the symbolical diagram in which each column is represented by a point and each row by a line joining the two points corresponding to the two columns in which it is represented. Thus for the evaluation of  $\kappa(3^4 2)$ , we have partitions represented by the figures



in each of which one angle at which two lines meet represents the column with only two entries. The reduction formula thus shows that the functions associated with these patterns will be each  $1/(n-1)$  of the functions associated with the simpler patterns represented by the three figures below (two of which are equivalent),



that is of those which occur in the evaluation of  $\kappa(3^4)$ . In respect of the numerical factor, too, it should be noticed that every way of setting up a partition for  $\kappa(3^4)$  corresponds to twelve ways of setting up one of the partitions for  $\kappa(3^4 2)$ , since any one of the six rows may be broken and connected in two ways with the elements of the new column. Thus a correspondence is established for the whole coefficient, and we have for the normal case

$$\kappa(3^4 2) = \frac{12}{n-1} \kappa(3^4) \kappa_2,$$

or, in general, since the number of rows in the partition is equal to the power of  $\kappa_2$  by which the coefficient is multiplied, the addition of a new part 2 is equivalent to the action of the operator

$$\frac{2\kappa_2^2}{n-1} \frac{d}{d\kappa_2},$$

an operator by means of which the higher cumulants of simultaneous distributions involving the estimated variance  $k_2$  may be very readily

obtained. In the multivariate case the operator for adding a variance or covariance  $k_{pq}$  is

$$\sum_{rs} \frac{1}{n-1} (\kappa_{pr} \kappa_{qs} + \kappa_{ps} \kappa_{qr}) \frac{d}{d\kappa_{rs}},$$

where  $\kappa_{pq}$  stands for the covariance of the variates  $p$  and  $q$ .

#### 4. Removal of a column of three entries.

The direct generalization of the method of the last section to partitions containing a column of three entries will evidently express the new function in terms of the functions of five simpler partitions, each obtained by suppressing the column of three entries, namely  $A$ , formed by the amalgamation of all three of the rows used in the column suppressed;  $B_1, B_2, B_3$ , formed by the amalgamation of two only of these three rows; and  $C$  by leaving all three rows distinct. In the new function the cofactor of  $1/n$  will evidently be  $A$ , that of  $-1/n(n-1)$  will be the sum of three quantities  $B-A$ , and that of  $2/n(n-1)(n-2)$  will be

$$C - (B_1 - A) - (B_2 - A) - (B_3 - A) - A = C - B_1 - B_2 - B_3 + 2A.$$

Hence the general formula is

$$\begin{aligned} A \left( \frac{1}{n} + \frac{3}{n(n-1)} + \frac{4}{n(n-1)(n-2)} \right) \\ - (B_1 + B_2 + B_3) \left( \frac{1}{n(n-1)} + \frac{2}{n(n-1)(n-2)} \right) + \frac{2C}{n(n-1)(n-2)} \\ \equiv \frac{n}{(n-1)(n-2)} A - \frac{1}{(n-1)(n-2)} (B_1 + B_2 + B_3) + \frac{2}{n(n-1)(n-2)} C. \end{aligned}$$

As an example we may derive the function for the pattern

$$\begin{array}{c} \times \times \times \\ \times \times \times \\ \times \times \times \\ \times \times . \end{array}$$

from those for the simpler patterns

$\begin{array}{c} \times \times \\ \times \times \end{array}$	$\begin{array}{c} \times \times \\ \times \times \\ \times \times \end{array}$	$\begin{array}{c} \times \times \\ \times \times \\ \times \times \\ \times \times \end{array}$
$A$	$B_1 = B_2 = B_3$	$C$
$\frac{1}{n-1}$	$\frac{n}{(n-1)(n-2)}$	$\frac{n(n+1)}{(n-1)(n-2)(n-3)}$

We have then

$$\frac{n}{(n-1)^3(n-2)} - \frac{8n}{(n-1)^2(n-2)^2} + \frac{2(n+1)}{(n-1)^2(n-2)^2(n-3)} \\ = \frac{n^3 - 8n^2 + 17n + 2}{(n-1)^3(n-2)^2(n-3)}.$$

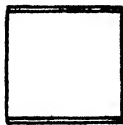
In its application to normal patterns the formula for removing a column of three entries is reduced to the simple formula

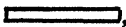
$$\frac{n}{(n-1)(n-2)} A,$$

but it should be noticed that  $A$  here is not necessarily the function of a normal pattern, for the other entries of the rows represented in the suppressed column may be in three different columns, in which case  $A$  will be the function of a pattern having a row with three entries. When, however, two of the amalgamated rows are duplicates joining the same pair of columns, the function will be expressed in terms of that of a normal pattern. In the representative diagram the effect of this will be to suppress a point, two of the lines from which are duplicates, and to replace the set of three lines meeting there by a single line joining their extremities



as does the broken line in the figure. By two such operations such a diagram as



is reduced to , representing the pattern



and having the function  $1/(n-1)$ , whence it easily follows that the diagram in question is associated with the function

$$n^2/(n-1)^3(n-2)^2.$$

In the case where three entries appear in the amalgamated row the partition of which  $A$  is the function, though not a normal one, is of the kind which must occur in the term in  $\kappa_3 \kappa_2^{r-1}$ , that is in the final term of the corresponding formula. Since any arrangement of a two-way partition having a marginal column represented by the partition  $(3 \ 2^{r-1})$  corresponds to six arrangements of the normal partition of the formula in which a new row of three entries has been added, these coefficients must be in the simple ratio

$$\frac{6n}{(n-1)(n-2)}.$$

For example, the coefficient of the term in  $\kappa_3 \kappa_2^2$  of the formula for  $\kappa(4 \ 3)$  is

$$\frac{36n}{(n-1)(n-2)},$$

whence it follows that the coefficient of the term in  $\kappa_2^5$  of the formula for  $\kappa(4 \ 3^2)$  must be

$$\frac{216n^2}{(n-1)^2(n-2)^2},$$

as may be verified from the formula given previously. Equally the corresponding coefficients in the formulae for  $\kappa(3 \ 2^2)$  and  $\kappa(3^2 \ 2^2)$  are

$$\frac{48}{(n-1)^2} \quad \text{and} \quad \frac{288n}{(n-1)^2(n-2)}.$$

All the normal terms occurring in the distribution of  $k_3$  and of its simultaneous distribution with other such statistics may then be obtained from the coefficients of simpler formulae.

##### 5. The removal of a column containing any number of entries.

In expressing the function of a pattern as a linear function of the patterns formed by deleting a column of  $r$  entries, and amalgamating the  $r$  corresponding rows into  $\rho'$  rows in accordance with the partition  $(p_1^{\pi_1} p_2^{\pi_2} \dots)$ , where

$$\sum p\pi = r,$$

$$\sum \pi = \rho',$$

let  $a(p_1^{\pi_1} p_2^{\pi_2} \dots)$  be the coefficient of the function of any one of the patterns so formed.

The functions consist of the sums of contributions from all possible separations of all the rows; we shall designate by  $Q(q_1^x q_2^x \dots)$  the total contribution of all such separations in which the  $r$  rows of the deleted column are separated in any particular way into  $\chi_1$  separates containing  $q_1$  of these rows each,  $\chi_2$  separates containing  $q_2$  of these rows each, and so on; then, if the partition  $(p_1^r p_2^r \dots)$  can be found by subdividing the parts of the partition  $(q_1^x q_2^x \dots)$  in  $\lambda$  ways, any  $Q$  will appear in the expansion with coefficient

$$\Sigma \lambda a(p_1^r p_2^r \dots),$$

the summation being over all partitions  $(p_1^r p_2^r \dots)$ , and this must be equated to its coefficient in the function of the whole pattern, namely

$$(-)^{\rho-1}(\rho-1)! \frac{(n-\rho)!}{n!},$$

where  $\Sigma(\chi) = \rho$ . We have in this way one such equation for every kind of partition  $(q_1^x q_2^x \dots)$ , and these are sufficient to determine the unknown coefficients  $a$ .

But if the statistic  $k_r$ , defined as having its mean sampling value equal to  $\kappa_r$ , be expanded in the form

$$k_r = \Sigma A(p_1^r p_2^r \dots) s_{p_1}^{r_1} s_{p_2}^{r_2} \dots,$$

where  $s_p$  is the sum of the  $p$ -th powers of the observations, then the coefficient of  $\mu_{q_1}^x \mu_{q_2}^x \dots$  in the mean value of the expansion, when the mean values of the  $s$ -products are expanded in terms of the moments  $\mu$ , by the general formula previously given (Fisher, *loc. cit.*, p. 207), will be

$$\Sigma \frac{n!}{(n-\rho)!} \mu A(p_1^r p_2^r \dots),$$

where  $\mu$  is the number of ways in which the parts of  $(p_1^r p_2^r \dots)$  may be amalgamated to form those of  $(q_1^x q_2^x \dots)$ , and this is to be equated to its known coefficient in the expansion of  $\kappa_r$ , namely

$$\frac{(-)^{\rho-1}(\rho-1)!}{\chi_1! \chi_2! \dots} \frac{r!}{(q_1!)^{\chi_1} (q_2!)^{\chi_2} \dots}.$$

The relation between  $\lambda$  and  $\mu$  may be found from the following consideration; the number of ways of dividing  $r$  objects into  $\rho'$  parts,  $p_1^r p_2^r \dots$ , these being grouped into  $\rho$  divisions  $q_1^x q_2^x \dots$ , may be obtained

either by multiplying by  $\lambda$  the number of ways of dividing  $r$  objects into parts  $q_1^{x_1} q_2^{x_2} \dots$ , i.e.

$$\frac{\lambda}{\chi_1! \chi_2! \dots} \frac{r!}{(q_1!)^{x_1} (q_2!)^{x_2} \dots},$$

or equally by multiplying by  $\mu$  the number of ways of dividing  $r$  objects into parts  $p_1^{\pi_1} p_2^{\pi_2} \dots$ , i.e.

$$\frac{\mu}{\pi_1! \pi_2! \dots} \frac{r!}{(p_1!)^{\pi_1} (p_2!)^{\pi_2} \dots}.$$

This relationship must hold for each particular separation of the partition  $(p_1^{\pi_1} p_2^{\pi_2} \dots)$  having specification  $(q_1^{x_1} q_2^{x_2} \dots)$ , as defined by MacMahon\*, or, as is here required, for all separations taken together. Consequently our second set of equations may be written

$$\Sigma \left\{ \lambda A(p_1^{\pi_1} p_2^{\pi_2} \dots) \div \frac{r!}{\pi_1! \pi_2! \dots (p_1!)^{\pi_1} (p_2!)^{\pi_2} \dots} \right\} = (-)^{\rho-1} (\rho-1)! \frac{(n-\rho)!}{n!},$$

showing that we can satisfy the equations for  $a$  by putting

$$a(p_1^{\pi_1} p_2^{\pi_2} \dots) = A(p_1^{\pi_1} p_2^{\pi_2} \dots) \div \frac{r!}{\pi_1! \pi_2! \dots (p_1!)^{\pi_1} (p_2!)^{\pi_2} \dots},$$

where the partition functions  $A$  have been already given up to partitions of six, that is, as far as is needed for the deletion of a column of six entries, in Fisher's expressions for  $k_1$  to  $k_6$  (*loc. cit.*, 203-4).

Let us illustrate this general method of proof by the case when  $r = 4$ . The function of a pattern containing a column of four entries is to be expressed in terms of the functions of the fifteen simpler patterns formed by deleting this column, and amalgamating the entries of the four rows in which it is represented in every possible way. We represent by  $F(4)$  the function of the patterns found by amalgamating all four rows into one, by  $F_1(31)$ ,  $F_2(31)$ ,  $F_3(31)$ ,  $F_4(31)$  the functions of the four patterns which can be found by leaving one row untouched and amalgamating into one the remaining three. Similarly there will be three functions  $F(2^2)$ , six functions  $F(21^2)$ , and one function  $F(1^4)$ . We have to find five coefficients  $a(4)$ ,  $a(31)$ ,  $a(2^2)$ ,  $a(21^2)$ , and  $a(1^4)$ , such that the new function shall be

$$a(4) F(4) + \sum_1^4 a(31) F(31) + \sum_1^3 a(2^2) F(2^2) + \sum_1^6 a(21^2) F(21^2) + a(1^4) F(1^4).$$

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\* P. A. MacMahon, *Combinatory analysis*, 1 (1915), 46.



Now the separations in which all four rows lie in different separates appear only in  $F(1^4)$ , and the sum of their contributions,  $Q(1^4)$ , comes into the new function with an additional factor

$$\frac{-6}{n(n-1)(n-2)(n-3)},$$

hence the first of the five equations required is

$$\alpha(1^4) = \frac{-6}{n(n-1)(n-2)(n-3)}.$$

Next any separation in which the four rows lie in three different separates will appear in one of the functions  $F(2\,1^2)$  and also in  $F(1^4)$ , and the sum of the contributions from such separations,  $Q(2\,1^2)$ , comes into the new function with the additional factor  $2/n(n-1)(n-2)$ , so that we have the second equation

$$\alpha(2\,1^2) + \alpha(1^4) = \frac{2}{n(n-1)(n-2)}.$$

Thirdly, any separation in which the rows fall two each into two different separates will appear in one of the functions  $F(2^2)$ , in two of the functions  $F(2\,1^2)$ , and in the function  $F(1^4)$ , so that

$$\alpha(2^2) + 2\alpha(2\,1^2) + \alpha(1^4) = \frac{-1}{n(n-1)}.$$

It should be noted that the coefficient 2 is the number of ways in which a partition  $(2^2)$  can be subdivided into a partition  $(2\,1^2)$ .

Fourthly, any separation in which three of the rows fall into one separate, and the remaining row into a different one, appears in one function  $F(3\,1)$ , in three functions  $F(2\,1^2)$ , and in the function  $F(1^4)$ , giving the equation

$$\alpha(3\,1) + 3\alpha(2\,1^2) + \alpha(1^4) = \frac{-1}{n(n-1)},$$

in which the coefficient 3 is the number of ways in which a partition  $(3\,1)$  may be subdivided into partitions  $(2\,1^2)$ .

Finally, any separation in which all four rows fall in the same separate appears in all the functions, so we have

$$\alpha(4) + 4\alpha(3\,1) + 3\alpha(2^2) + 6\alpha(2\,1^2) + \alpha(1^4) = \frac{1}{n},$$

completing the set of five equations necessary to determine the coefficients  $\alpha$ .

The equations may evidently be solved by direct substitution, yielding the solution

$$a(1^4) = \frac{-6}{n(n-1)(n-2)(n-3)},$$

$$a(2 \ 1^3) = \frac{2}{(n-1)(n-2)(n-3)},$$

$$a(2^2) = \frac{-1}{(n-2)(n-3)},$$

$$a(3 \ 1) = \frac{-(n+1)}{(n-1)(n-2)(n-3)},$$

$$a(4) = \frac{n(n+1)}{(n-1)(n-2)(n-3)}.$$

The proof of the relationship under discussion depends, however, upon the correspondence of the equations for  $a$  with those obtained by putting

$$k_4 = A(4)s_4 + A(3 \ 1)s_3s_1 + A(2^2)s_2^2 + A(2 \ 1^3)s_2s_1^2 + A(1^4)s_1^4,$$

and expressing the conditions that the mean value of the statistic so constructed shall be equal to the population parameter  $\kappa_4$  with its identical expression in terms of the moments

$$\kappa_4 = \mu_4 - 4\mu_3\mu_1 - 3\mu_2^2 + 12\mu_2\mu_1^2 - 6\mu_1^4.$$

Here  $\mu_1^4$  will appear in the expansion of the mean value of  $s_1^4$  only, giving

$$n(n-1)(n-2)(n-3)A(1^4) = -6;$$

$\mu_2\mu_1^2$  will appear in the means of  $s_2s_1^2$  and of  $s_1^4$ , giving

$$n(n-1)(n-2)\{A(2 \ 1^3) + 6A(1^4)\} = 12;$$

$\mu_2^2$  will appear in the means of  $s_2^2$ ,  $s_2s_1^2$ , and  $s_1^4$ , giving

$$n(n-1)\{A(2^2) + A(2 \ 1^2) + 3A(1^4)\} = -3,$$

while the two remaining equations are

$$n(n-1)\{A(3 \ 1) + 2A(2 \ 1^2) + 4A(1^4)\} = -4$$

and  $n\{A(4) + A(3 \ 1) + A(2^2) + A(2 \ 1^2) + A(1^4)\} = 1,$

the numerical factors in these equations being the number of ways in which the parts of any particular partition may be amalgamated to form those of the partition in the equation of which it occurs.

These equations also may be solved by direct substitution, giving the familiar relations

$$A(1^4) = \frac{-6}{n(n-1)(n-2)(n-3)},$$

$$A(2 \ 1^3) = \frac{12}{(n-1)(n-2)(n-3)},$$

$$A(2^2) = \frac{-8}{(n-2)(n-3)},$$

$$A(3 \ 1) = \frac{-4(n+1)}{(n-1)(n-2)(n-3)},$$

$$A(4) = \frac{n(n+1)}{(n-1)(n-2)(n-3)},$$

from which it is evident that we can obtain the corresponding solutions for  $\alpha$  by dividing by the number of ways in which four objects may be distributed in the required partition, or in general by

$$\frac{1}{\pi_1! \pi_2! \dots} \frac{r!}{(p_1!)^{\pi_1} (p_2!)^{\pi_2} \dots}.$$

#### 6. Proof of the vanishing of a class of zero functions.

The fact that the function of a two-way partition can be found by an expression linear in the functions of partitions with one less column, and with the rows represented in the deleted column more or less amalgamated, may be used to prove that the partition function is necessarily zero in a class of cases in which its vanishing has hitherto only been noted empirically. The class in question is that of partitions the rows of which can be divided into two groups, which, whatever may be their internal connections, are only connected with each other by entries in a single column. For example, in the pattern

$$\begin{array}{cccc} \times & \times & \times & . \\ \times & \times & \times & . \\ . & . & \times & \times \\ . & . & \times & \times \end{array}$$

the group of columns represented in the two upper rows and the group represented in the two lower rows have in common only a single column, namely the third from the left. Evidently all patterns in which any row has only one entry belong to this class, but, whereas a statistical reason

## MISCELLANEA

## MATHEMATICAL THEOREMS INVOLVED IN THE ANALYSIS OF VARIANCE.

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IN this paper proofs are given of the essential theorems involved in the "Analysis of Variance" method which R. A. Fisher has invented. An endeavour has been made to treat them in an elementary manner, so as to make them available in one place for the mathematical-statistical student of average ability, or any others interested in the subject. For this reason somewhat full proofs have been given and points dealt with in detail which will doubtless appear obvious to the highly trained mathematical statistician. No claim is made to originality, except perhaps in Sections III and IV, where two results are given which may conceivably be new.

I. *Distribution of Estimates of Variance and their Ratios.*

Suppose we have a hypothetically infinite population in which a variate  $x$  is normally distributed with mean  $m$  and variance  $\sigma^2$ . Then in a random sample of  $n'$  individuals the chance of getting a set of values  $x_1, x_2 \dots x_{n'}$  in the interval  $dx_1 dx_2 \dots dx_{n'}$ , is

$$df = \left( \frac{1}{\sqrt{2\pi\sigma}} \right)^{n'} e^{-\frac{1}{2} \sum_{r=1}^{n'} \left( \frac{x_r - m}{\sigma} \right)^2} dx_1 dx_2 \dots dx_{n'} \quad (1)$$

(i)  $x_1, x_2 \dots x_{n'}$  are here supposed all independent.

Let  $\bar{x}$  be the sample mean, i.e.  $\bar{x} = \frac{1}{n'} \sum_{r=1}^{n'} x_r$ .

Let  $s^2$  be given by  $s^2 = \frac{1}{n' - 1} \sum_{r=1}^{n'} (x_r - \bar{x})^2$ .

Since  $\sum_{r=1}^{n'} (x_r - m)^2 = \sum_{r=1}^{n'} (x_r - \bar{x})^2 + n'(\bar{x} - m)^2$ , (1) may be written

$$\begin{aligned} df &= \left( \frac{1}{\sqrt{2\pi\sigma}} \right)^{n'} e^{-\frac{n'(\bar{x} - m)^2}{2\sigma^2}} e^{-\frac{1}{2} \sum_{r=1}^{n'} \left( \frac{x_r - \bar{x}}{\sigma} \right)^2} dx_1 \dots dx_{n'} \\ &= \left( \frac{1}{\sqrt{2\pi\sigma}} \right)^{n'} e^{-\frac{n'(\bar{x} - m)^2}{2\sigma^2}} e^{-\frac{(n'-1)s^2}{2\sigma^2}} dx_1 \dots dx_{n'} \quad (2) \end{aligned}$$

We must now convert the differential element  $dx_1 dx_2 \dots dx_{n'}$ .

Suppose in space of  $n'$  dimensions we take through a point  $O$ ,  $n'$  axes mutually at right angles, then using these  $n'$  lines as co-ordinate axes we may represent our sample point  $P$  by the co-ordinates  $x_1, x_2 \dots x_{n'}$ .

The equation  $\bar{x} = \frac{1}{n'} (x_1 + x_2 + \dots + x_{n'})$  may be interpreted as meaning that for a given value of  $\bar{x}$ ,  $P$  lies in the hyper-plane

$$x_1 + x_2 + \dots + x_{n'} = n'\bar{x}.$$

If  $M$  be the foot of the perpendicular from  $O$  on to this plane

$$OM = \bar{x}\sqrt{n'}$$

and 
$$PM = \sqrt{\sum_{r=1}^{n'} (x_r - \bar{x})^2} = s\sqrt{n' - 1}.$$

We may thus use  $OM$ ,  $PM$  and  $n' - 2$  independent angular functions as generalised cylindrical co-ordinates of the point  $P$ , and obtain

$$dx_1 dx_2 \dots dx_{n'} = f_1(\theta_1) f_2(\theta_2) \dots f_{n'-2}(\theta_{n'-2}) s^{n'-2} d\bar{x} ds.$$

The reason for the exponent  $(n' - 2)$  of  $s$  in this expression is that for a constant value of  $s$ ,  $P$  lies on a hyper-sphere with centre  $M$ , the dimensions of whose surface are  $n' - 2$ . (This may easily be seen by analogy from the case of samples of 3, when  $P$  lies on a circle.)

The angular functions, being entirely independent of  $\bar{x}$  and  $s$ , will, when integrated over the whole range of their possible values, give rise simply to a constant; thus we have

$$df = C e^{-\frac{n'(\bar{x}-m)^2}{2\sigma^2}} e^{-\frac{(n'-1)s^2}{2\sigma^2}} s^{n'-2} d\bar{x} ds \dots \quad (3)$$

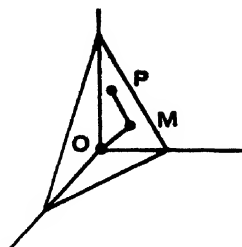
Thus the distribution of  $\bar{x}$  and  $s$  are quite independent, that of  $s$  being

$$df = K \left( \frac{1}{\sigma} \right)^{n'-2} e^{-\frac{(n'-1)s^2}{2\sigma^2}} \frac{ds}{\sigma} \dots \quad (4)$$

By integration we may show that

$$K = \frac{(n' - 1)^{\frac{n'-1}{2}}}{2^{\frac{n'-3}{2}}} \frac{1}{\Gamma\left(\frac{n' - 1}{2}\right)} \dots \quad (5)$$

It follows that the mean value of  $s^2$  in all possible samples is  $\sigma^2$ .  $s$  is also the value of  $\sigma$  obtained by applying the method of maximum-likelihood to (4).  $s^2$  is thus an unbiased estimate of  $\sigma^2$ ; it may also be shown to be efficient; that is, no other estimate of  $\sigma^2$  can have a smaller sampling variance. We call  $s^2$  an estimate of  $\sigma^2$ , based on  $n' - 1$  degrees of freedom.



The essential reason why  $n' - 1$  and not  $n'$  is our correct divisor of  $S(x - \bar{x})^2$ , in order to get an unbiased estimate of  $\sigma^2$ , is that our estimate of the mean in this expression has also been made from the sample.

We can get a clearer idea of why this is so if we suppose that we had a previous knowledge of  $m$ , the mean of the population; then we might make an estimate of  $\sigma^2$  from  $S(x_r - m)^2$ ; the correct divisor would now be  $n'$ . For consider the expression (1) and let

$$s' = \frac{1}{n'} S(x_r - m)^2.$$

Suppose now our co-ordinates are the deviations from the fixed population mean.

The point  $P$  now lies on a hyper-sphere the dimensions of whose surface are  $n' - 1$ ; therefore when we transform the differential element  $dx_1 dx_2 \dots dx_{n'}$  into polar co-ordinates we obtain

$$dx_1 dx_2 \dots dx_{n'} = f_1(\theta_1) f_2(\theta_2) \dots f_{n'-1}(\theta_{n'-1}) s'^{n'-1} ds'$$

and the distribution of  $s'$  is easily seen to be

$$df = Ke^{-\frac{n's'^2}{2\sigma^2}} \left(\frac{s'}{\sigma}\right)^{n'-1} \frac{ds'}{\sigma} \dots \dots (6)$$

which is the same result as (4) with  $n'$  instead of  $n' - 1$ . Our unbiased and efficient estimate would now be  $S \frac{(x_r - m)^2}{n'}$ .

(ii) Now let us suppose that in addition to the relation between the sample mean and its individual values, there are  $p$  independent relations between  $x_1, x_2 \dots x_{n'}$ , and now let  $s^2 = \frac{1}{n' - p - 1} S(x - \bar{x})^2$ . The point  $P$  which represents our sample now lies not only in the hyper-plane

$$n'\bar{x} = x_1 + x_2 + \dots + x_{n'}$$

but in  $p$  other hyper-planes. The result will be that for a fixed value of  $s$ ,  $P$  lies on a hyper-sphere, with centre  $M$ , the dimensions of whose surface are now only  $n' - p - 2$ . Otherwise the argument is exactly the same as before.

The distribution of  $S$  is therefore now

$$df = Ke^{-\frac{(n'-p-1)s^2}{2\sigma^2}} \left(\frac{s}{\sigma}\right)^{n'-p-2} \frac{ds}{\sigma}$$

where 
$$K = \frac{(n' - p - 1)^{\frac{n'-p-1}{2}}}{2^{\frac{n'-p-3}{2}}} \frac{1}{\Gamma\left(\frac{n' - p - 1}{2}\right)} \dots (7)$$

and  $s^2$  is an unbiased and efficient estimate of  $\sigma^2$ .

We may sum up these results by saying that if  $n$  is our number of degrees of freedom and if  $s^2$  is given by

$$s^2 = \frac{1}{n} \sum_{r=1}^{n'} (x_r - \bar{x})^2$$

$n'$  being the number of our observations,  $s^2$  is our unbiased and efficient estimate of  $\sigma^2$ , and the distribution of  $s$  given by

$$df = \frac{n^{\frac{n}{2}}}{2^{\frac{n-2}{2}} \Gamma\left(\frac{n}{2}\right)} e^{-\frac{ns^2}{2\sigma^2}} \left(\frac{s}{\sigma}\right)^{n-1} \frac{ds}{\sigma}$$

We notice that the number of degrees of freedom is equal to the number of observations made, less the number of independent relations between them, account being taken of the fact that the population mean is itself estimated from the sample.

(iii) Suppose now we have two estimates of the same variance,  $s_1^2$  and  $s_2^2$ , based respectively on  $n_1$  and  $n_2$  degrees of freedom.

Let  $\omega = \frac{s_1}{s_2}$ , then  $s_1 = s_2 \omega$

The distribution of  $s_1$  is given by

$$\begin{aligned} df &= \frac{n_1^{\frac{n_1}{2}}}{2^{\frac{n_1-2}{2}} \Gamma\left(\frac{n_1}{2}\right)} e^{-\frac{n_1 s_1^2}{2\sigma^2}} \left(\frac{s_1}{\sigma}\right)^{n_1-1} \frac{ds_1}{\sigma} \\ &= \frac{n_1^{\frac{n_1}{2}}}{2^{\frac{n_1-2}{2}} \Gamma\left(\frac{n_1}{2}\right)} e^{-\frac{n_1 \omega^2 s_2^2}{2\sigma^2}} \frac{(\omega s_2)^{n_1-1}}{\sigma^{n_1}} s_2 d\omega \quad \dots \quad (8) \end{aligned}$$

for a given value of  $s_2$ .

But the distribution of  $s_2$  is given by

$$df = \frac{n_2^{\frac{n_2}{2}}}{2^{\frac{n_2-2}{2}} \Gamma\left(\frac{n_2}{2}\right)} e^{-\frac{n_2 s_2^2}{2\sigma^2}} \left(\frac{s_2}{\sigma}\right)^{n_2-1} \frac{ds_2}{\sigma} \quad \dots \quad (9)$$

Therefore to obtain the distribution of  $\omega$  we need only to multiply (8) by (9) and integrate for  $s_2$  from 0 to  $\infty$ .

We obtain :—

$$df = d\omega \int_0^\infty \frac{n_1^{\frac{n_1}{2}} n_2^{\frac{n_2}{2}}}{2^{\frac{n_1+n_2-2}{2}} \Gamma\left(\frac{n_1}{2}\right) \Gamma\left(\frac{n_2}{2}\right)} e^{-\frac{(n_1 \omega^2 + n_2) s_2^2}{2\sigma^2}} \frac{s_2^{n_1+n_2-1} \omega^{n_1-1}}{\sigma^{n_1+n_2}} ds_2$$

$$= \frac{2n_1^{\frac{n_1}{2}} n_2^{\frac{n_2}{2}} \Gamma\left(\frac{n_1 + n_2}{2}\right)}{\Gamma\left(\frac{n_1}{2}\right) \Gamma\left(\frac{n_2}{2}\right)} \frac{\omega^{n_1-1} d\omega}{(n_1 \omega^2 + n_2)^{\frac{n_1+n_2}{2}}}$$

If now  $z = \log \omega = \log \frac{s_1}{s_2}$

we have for the distribution of  $z$

$$df = \frac{2n_1^{\frac{n_1}{2}} n_2^{\frac{n_2}{2}} \Gamma\left(\frac{n_1 + n_2}{2}\right)}{\Gamma\left(\frac{n_1}{2}\right) \Gamma\left(\frac{n_2}{2}\right)} \frac{e^{n_1 z} dz}{(n_1 e^{2z} + n_2)^{\frac{n_1+n_2}{2}}} \quad \dots \quad (10)$$

The 5 per cent. and 1 per cent. points of this distribution have been tabulated by Fisher and are used to test whether these two estimates of the same variance are significantly different.

It may be remarked here that if  $s_1^2$  and  $s_2^2$  are estimates of two different variances  $\sigma_1^2$  and  $\sigma_2^2$ , and  $\zeta = \log \frac{\sigma_1^2}{\sigma_2^2}$ , it may be shown in exactly the same way that  $z - \zeta$  has this distribution.

## II. *The Structure of the Analysis of Variance and its Use as a Significance Test.*

(i) Suppose that we have a random sample of  $N(=rs)$  values from a homogeneous normal population with variance  $\sigma^2$ , and that these values be subdivided into  $s$  classes with  $r$  individuals in each class. Let  $x_{uv}$  denote the  $u$ th individual in the  $v$ th class,  $\bar{x}_{.v}$  the mean of the  $v$ th class,  $\bar{x}_u$  the mean of the  $u$ th individuals in all classes,  $\bar{x}$  the general mean. Then we have the identity

$$S(x_{uv} - \bar{x})^2 = S(\bar{x}_{.v} - \bar{x})^2 + S(\bar{x}_u - \bar{x})^2 + S(x_{uv} - \bar{x}_{.v} - \bar{x}_u + \bar{x})^2 \quad \dots \quad (11)$$

Where the symbol  $S$  is used to denote summation over all the individuals in the sample.

Then, as we have seen,  $\frac{S(x_{uv} - \bar{x})^2}{rs - 1}$  is an unbiased estimate of  $\sigma^2$  based on  $rs - 1$  degrees of freedom, whose distribution in random samples we have already found.

(ii) Now consider  $S(\bar{x}_{.v} - \bar{x})^2$ .

Let  $E(w)$  denote the mathematical expectation of  $w$  or, as we may say, its mean value in all possible samples. Then if  $m$  be the population mean,

$$E(\bar{x}_{.v} - \bar{x})^2 = E \left[ \frac{1}{r} \sum_{u=1}^r (x_{uv} - m) - (\bar{x} - m) \right]^2$$



$$= E \left[ \frac{1}{r^2} \sum_{u=1}^r (x_{uv} - m)^2 - \frac{2(\bar{x} - m)}{r} \sum_{u=1}^r (x_{uv} - m) + (\bar{x} - m)^2 \right].$$

But  $E(x_{uv} - m)^2 = \sigma^2$

$$E(x - m)(x' - m) = 0$$

where  $x$  and  $x'$  are two different sample values,

whence  $E(\bar{x} - m)(x_{uv} - m) = \frac{\sigma^2}{rs}$

$$E(\bar{x} - m)^2 = \frac{\sigma^2}{rs}$$

Thus 
$$E(\bar{x}_{..} - \bar{x})^2 = \frac{\sigma^2}{r} - \frac{2\sigma^2}{rs} + \frac{\sigma^2}{rs}$$

$$= \frac{\sigma^2}{r} \left( \frac{s-1}{s} \right)$$

and  $ES(\bar{x}_{..} - \bar{x})^2 = \sigma^2(s-1) \dots \dots \dots (12)$

Thus  $\frac{S(\bar{x}_{..} - \bar{x})^2}{s-1}$  is an unbiased estimate of  $\sigma^2$ .

Now we know that  $x$  is normally distributed, therefore  $\bar{x}_{..}$ , which merely depends on the sum of a number of  $x$ 's, will also be normally distributed, therefore the distribution of  $\frac{S(\bar{x}_{..} - \bar{x})^2}{s-1}$  will be given by the previous theory. This quantity is in fact an estimate of  $\sigma^2$  based on  $(s-1)$  degrees of freedom. It is, of course, obvious that there are  $(s-1)$  degrees of freedom because we have  $s$  class means, and our estimate  $\bar{x}$  of the general mean has been made from the sample, by which process one degree of freedom is lost.

(iii) In exactly the same way we may show that  $\frac{S(\bar{x}_{u.} - \bar{x})^2}{r-1}$  is an unbiased estimate of  $\sigma^2$  based on  $(r-1)$  degrees of freedom, whose distribution is given by the previous theory.

(iv) Now consider  $S(x_{uv} - \bar{x}_{..} - \bar{x}_{u.} + \bar{x})^2$ .

We have 
$$E(x_{uv} - \bar{x}_{..} - \bar{x}_{u.} + \bar{x})^2$$

$$= E\{(x_{uv} - m) - (\bar{x}_{..} - m) - (\bar{x}_{u.} - m) + (\bar{x} - m)\}^2$$

$$= E\{(x_{uv} - m)^2 + (\bar{x}_{..} - m)^2 + (\bar{x}_{u.} - m)^2 + (\bar{x} - m)^2$$

$$- 2(x_{uv} - m)(\bar{x}_{..} - m) - 2(x_{uv} - m)(\bar{x}_{u.} - m)$$

$$+ 2(x_{uv} - m)(\bar{x} - m) - 2(\bar{x}_{..} - m)(\bar{x} - m)$$

$$- 2(\bar{x}_{u.} - m)(\bar{x} - m) + 2(\bar{x}_{..} - m)(\bar{x}_{u.} - m)\}$$

But remembering  $E(x_{uv} - m)^2 = \frac{\sigma^2}{rs}$

and  $E(x - m)(x' - m) = 0$  as before, we easily find

$$E(\bar{x}_{..} - m)^2 = \frac{\sigma^2}{r}$$

$$E(\bar{x}_u - m)^2 = \frac{\sigma^2}{s}$$

$$E(\bar{x} - m)^2 = \frac{\sigma^2}{rs}$$

$$E(x_{uv} - m)(\bar{x}_v - m) = \frac{\sigma^2}{r}$$

$$E(x_{uv} - m)(\bar{x}_u - m) = \frac{\sigma^2}{s}$$

$$E(x_{uv} - m)(\bar{x} - m) = \frac{\sigma^2}{rs}$$

$$E(\bar{x}_v - m)(\bar{x} - m) = \frac{\sigma^2}{rs}$$

$$E(\bar{x}_u - m)(\bar{x} - m) = \frac{\sigma^2}{rs}$$

$$E(\bar{x}_v - m)(\bar{x}_u - m) = \frac{\sigma^2}{rs}$$

Whence 
$$E(x_{uv} - \bar{x}_v - \bar{x}_u + \bar{x})^2 = \sigma^2 \left( 1 - \frac{1}{r} - \frac{1}{s} + \frac{1}{rs} \right)$$
  

$$= \frac{\sigma^2(r-1)(s-1)}{rs} \quad (13)$$

Therefore  $\frac{ES(x_{uv} - \bar{x}_v - \bar{x}_u + \bar{x})^2}{(r-1)(s-1)}$  is an unbiased estimate of  $\sigma^2$  whose distribution is given by the previous theory; \* for the sums or differences of quantities which are normally distributed are themselves normally distributed.

It is easy to see that there are  $(r-1)(s-1)$  degrees of freedom for the  $rs$  quantities,

$$X_{uv} = x_{uv} - \bar{x}_v - \bar{x}_u + \bar{x} \quad \left. \begin{array}{l} u = 1 \dots r \\ v = 1 \dots s \end{array} \right\}$$

for if these quantities are written down in  $s$  rows with  $r$  in a row thus :

$$\begin{array}{ccccccc} X_{11}, & X_{21} & \dots & \dots & \dots & X_{r1} \\ X_{12}, & X_{22} & \dots & \dots & \dots & X_{r2} \\ \dots & \dots & \dots & \dots & \dots & \dots \\ X_{1s}, & X_{2s} & \dots & \dots & \dots & X_{rs} \end{array}$$

it is at once seen that the sums of all the rows and columns are zero. Thus  $(s-1)$  of the numbers in each of the first  $r-1$  columns can be given any values we please and the remaining ones are then

\* The reader should here be referred to "Applications of Student's Distribution" by R. A. Fisher, *Metron*, Vol. V, No. 3, p. 10, 1925, where a general proof is given of a theorem of which this and all similar theorems are particular cases.

determinate. Thus there are only  $(r-1)(s-1)$  independent quantities in the set.

(v) To sum up, we have shown: (a) that the total sum of squares,  $S(x_{uv} - \bar{x})^2$ , may be divided up in accordance with the following scheme:

	Sum of Squares.	Degrees of Freedom.
(1) Between class means ...	$S(\bar{x}_{.v} - \bar{x})^2$	$s - 1$
(2) Between means of corresponding individuals in different classes ...	$S(\bar{x}_{u.} - \bar{x})^2$	$r - 1$
(3) Interaction between (1) and (2) ... ..	$S(x_{uv} - \bar{x}_{.v} - \bar{x}_{u.} + \bar{x})^2$	$(r-1)(s-1)$
Total ... ..	$S(x_{uv} - \bar{x})^2$	$rs - 1$

(b) With each of these expressions (1), (2) and (3) may be associated the corresponding number of degrees of freedom. These are always equal to the number of expressions squared, less the number of independent relations between them.

(c) If our sample is a random sample from a homogeneous normal population with variance  $\sigma^2$ , then when each of the expressions (1), (2) and (3) is divided by its corresponding degrees of freedom we obtain an unbiased estimate of  $\sigma^2$ . This estimate will be distributed in random samples in the manner shown in Section I (ii). The ratio "z" of any two such estimates will be distributed in the manner shown in Section I (iii).

(vi) This procedure may be used as a *test of significance* to test whether our sample is a random sample from a homogeneous normal population or not. If any two of our estimates of variance are very unequal, they will give rise to a large value of "z." From the known distribution of "z" we can calculate the probability of such a large value occurring in a random sample from a homogeneous normal population.

If this probability is too small we reject the hypothesis that the sample is a random sample from a homogeneous normal population.

For example, in this particular instance we may regard our  $s$  classes as being  $s$  blocks of land, and corresponding individuals in different classes as being plot yields, one in each of the different blocks, receiving like manurial treatments. The expression (1) is then  $r$  times the sum of the squares of the deviations of the block means from the general mean. The expression (2) is  $s$  times the sum of the squares of the deviations of the treatment means from the general mean. Expression (3) is what has come to be known as "*sum of squares due to error.*"

We may compare (1) and (3) by means of the "z" test. If (1) is significantly larger than (3) as judged by this test, we conclude that the soil is heterogeneous.

If (2) is significantly larger than (3) as judged by the "z" test, we conclude that our manurial treatments are significantly affecting yield.

In either of these two cases the hypothesis that the sample is a random one from a homogeneous normal population is definitely disproved. In actual practice when this hypothesis has been disproved, it is the custom to use the estimate of variance obtained on dividing the sum of squares in expression (3) by the corresponding degrees of freedom as the basis for calculating the standard errors of the treatment means. This process is, in fact, theoretically correct, but it must be noted here that it requires further theoretical justification. We have, once we have established that our sample is not a random one from a homogeneous normal population, to consider very carefully what is the nature of the population from which we are in fact sampling.

(vii) The properties demonstrated in the previous sections may easily be generalized. We might, for instance, suppose that we have  $N = rst$  observed values divided into  $t$  classes. Each of these classes might be divided into  $s$  sub-classes with  $r$  individuals in each.

Let  $x_{uvw}$  be the value of the variate for the  $u$ th individual in the  $v$ th sub-class in the  $w$ th class,  $\bar{x}$  the general mean,  $\bar{x}_{u..}$  the mean of all the  $u$ th individuals,  $\bar{x}_{..v}$  the mean of all the individuals in the  $v$ th sub-classes,  $\bar{x}_{..w}$  the mean of all the individuals in the  $w$ th class,  $\bar{x}_{uv}$  the mean of all the individuals in the  $u$ th class and  $v$ th sub-class, with similar meanings for  $\bar{x}_{.vw}$  and  $\bar{x}_{u.w}$ . Then we can show

$$\begin{aligned}
 S(x_{uvw} - \bar{x})^2 &= S(\bar{x}_{u..} - \bar{x})^2 + S(\bar{x}_{..v} - \bar{x})^2 + S(\bar{x}_{..w} - \bar{x})^2 \\
 &\quad + S(\bar{x}_{uv} - \bar{x}_{u..} - \bar{x}_{..v} + \bar{x})^2 \\
 &\quad + S(\bar{x}_{u.w} - \bar{x}_{u..} - \bar{x}_{..w} + \bar{x})^2 \\
 &\quad + S(\bar{x}_{.vw} - \bar{x}_{..v} - \bar{x}_{..w} + \bar{x})^2 \\
 &\quad + S(x_{uvw} - \bar{x}_{uv} - \bar{x}_{.vw} - \bar{x}_{u.w} \\
 &\quad \quad + \bar{x}_{u..} + \bar{x}_{..v} + \bar{x}_{..w} - \bar{x})^2 \quad . \quad . \quad (14)
 \end{aligned}$$

the summation being in each case extended over *every individual* in the sample.

It may also be shown that the mathematical expectations of each of the seven terms in the above expression are as follows :

- (1)  $(r - 1)\sigma^2$
- (2)  $(s - 1)\sigma^2$
- (3)  $(t - 1)\sigma^2$
- (4)  $(r - 1)(s - 1)\sigma^2$
- (5)  $(s - 1)(t - 1)\sigma^2$
- (6)  $(t - 1)(r - 1)\sigma^2$
- (7)  $(r - 1)(s - 1)(t - 1)\sigma^2$

The degrees of freedom are in each case the coefficients of  $\sigma^2$ . It is easily shown that their total is  $rst - 1$ , or the number of degrees of freedom appropriate to  $S(x_{uvw} - \bar{x})^2$ .

Any of these seven estimates of variance may be compared with any other by the "z" test, to test the hypothesis whether the sample is a random sample from a homogeneous normal population.

### III. *Estimation in Cases where Heterogeneity has already been Shown.*

We must now return to the point mentioned in Section II (vi). Suppose we have by the application of the significance test shown that our sample cannot be regarded as a random sample from a homogeneous normal population. We have to justify the use of the "error term" in the analysis of variance as the basis of our estimate of the standard errors of the means in which we are interested. (In our example these were the treatment means in an agricultural experiment.)

This involves a careful consideration of the nature of the population from which we must now regard our sample as coming.

We shall start by making the simplest possible hypothesis about this population, namely, that it may be divided into  $s$  groups with  $r$  sub-groups within each group. We may, if we wish, regard the groups as corresponding to blocks of land and the sub-groups as manurial treatments.

Let  $x_{uv}$  be a value of the variate in the  $u$ th sub-group of the  $v$ th group, let  $m_{uv}$  be the mean value of  $x_{uv}$  for all individuals in the population who are in the  $u$ th sub-group of the  $v$ th group.

Then we have  $x_{uv} = m_{uv} + \xi_{uv}$ , where the mean value of  $\xi_{uv}$  over the sub-group is zero. We shall suppose our sample to be composed by taking one individual at random out of each sub-group.

In accordance with our previous notation, we shall use  $m_u$  to denote the mean value in the population of the  $s$  values of  $m_{uv}$  in the  $u$ th sub-groups,  $m_v$  to denote the mean of the  $r$  values of  $m_{uv}$  in the  $v$ th group, and finally  $m$  to denote the population mean.  $\bar{x}_u$ ,  $\bar{x}_v$  and  $\bar{x}$  are as before the corresponding quantities in the sample. We shall suppose that the variance of the individuals in any one "cell" ( $uv$ ) is  $\sigma^2$  and that this is the same for all cells.

Then we have

$$\begin{aligned} E(\bar{x}_u - \bar{x})^2 &= E(m_u - m + \bar{\xi}_u - \bar{\xi})^2 \\ &= (m_u - m)^2 + E(\bar{\xi}_u - \bar{\xi})^2 \end{aligned}$$

since the mean value of the product term in all possible samples is easily seen to be zero. But

$$E(\xi_{u.}^2) = E\frac{1}{s^2}(\xi_{u_1} + \xi_{u_2} + \dots + \xi_{u_s})^2 = \frac{\sigma^2}{s}$$

$$E(\xi_{u.} \xi) = E\frac{1}{rs^2}(\xi_{u_1} + \xi_{u_2} + \dots + \xi_{u_s})S(\xi) = \frac{\sigma^2}{rs}$$

$$E(\xi^2) = \frac{\sigma^2}{rs}$$

$$E(\xi_{u.} - \xi)^2 = \frac{\sigma^2}{s} - \frac{\sigma^2}{rs} = \frac{\sigma^2}{s} \left( \frac{r-1}{r} \right)$$

$$\therefore ES(\bar{x}_{u.} - \bar{x})^2 = S(m_{u.} - m)^2 + (r-1)\sigma^2 \quad (14)$$

This may be written in the alternative form

$$sE \sum_{u=1}^r (\bar{x}_{u.} - \bar{x})^2 = s \sum_{u=1}^r (m_{u.} - m)^2 + (r-1)\sigma^2. \quad (14 \text{ bis})$$

In precisely the same way we can show

$$ES(\bar{x}_{.v} - \bar{x})^2 = S(m_{.v} - m)^2 + (s-1)\sigma^2 \quad (15)$$

or alternatively

$$rE \sum_{v=1}^s (\bar{x}_{.v} - \bar{x})^2 = r \sum_{v=1}^s (m_{.v} - m)^2 + (s-1)\sigma^2. \quad (15 \text{ bis})$$

The meaning of equations (14) and (15) will become clearer when we have considered the "error" term. We have:—

$$\begin{aligned} & E(x_{uv} - \bar{x}_{u.} - \bar{x}_{.v} + \bar{x})^2 \\ &= E(m_{uv} - m_{u.} - m_{.v} + m + \xi_{uv} - \bar{\xi}_{u.} - \bar{\xi}_{.v} + \bar{\xi})^2 \\ &= (m_{uv} - m_{u.} - m_{.v} + m)^2 + E(\xi_{uv} - \bar{\xi}_{u.} - \bar{\xi}_{.v} + \bar{\xi})^2. \quad (16) \end{aligned}$$

the mean value of the product term in all possible samples being easily shown to vanish as before.

$$\begin{aligned} \text{Now} \quad & E(\xi_{uv} - \bar{\xi}_{u.} - \bar{\xi}_{.v} + \bar{\xi})^2 \\ &= E(\xi_{uv}^2 + \bar{\xi}_{u.}^2 + \bar{\xi}_{.v}^2 + \bar{\xi}^2 - 2\xi_{uv}\bar{\xi}_{u.} - 2\xi_{uv}\bar{\xi}_{.v} \\ &\quad + 2\xi_{uv}\bar{\xi} + 2\bar{\xi}_{u.}\bar{\xi}_{.v} - 2\bar{\xi}_{u.}\bar{\xi} - 2\bar{\xi}_{.v}\bar{\xi}) \end{aligned}$$

But it is easily shown that

$$E\xi_{uv}^2 = \sigma^2 \qquad E\xi_{uv}\bar{\xi}_{u.} = \frac{\sigma^2}{s}$$

$$E\bar{\xi}_{u.}^2 = \frac{\sigma^2}{s} \qquad E\xi_{u.}\bar{\xi}_{.v} = \frac{\sigma^2}{r}$$

$$E\bar{\xi}_{.v}^2 = \frac{\sigma^2}{r} \qquad E\xi_{uv}\bar{\xi} = \frac{\sigma^2}{rs}$$

$$E\bar{\xi}^2 = \frac{\sigma^2}{rs} \qquad E\bar{\xi}_{u.}\bar{\xi}_{.v} = \frac{\sigma^2}{rs}$$

$$E\bar{\xi}_{u.}\bar{\xi} = E\bar{\xi}_{.v}\bar{\xi} = \frac{\sigma^2}{rs}$$

Thus 
$$E(\xi_{uv} - \bar{\xi}_{u.} - \bar{\xi}_{.v} + \bar{\xi})^2 = \frac{\sigma^2(r-1)(s-1)}{rs}$$

and 
$$ES(x_{uv} - \bar{x}_{u.} - \bar{x}_{.v} + \bar{x})^2 = S(m_{uv} - m_{u.} - m_{.v} + m)^2 + (r-1)(s-1)\sigma^2 \quad (17)$$

This is as far as we can get without making a further assumption. We now assume that  $x_{uv}$  is made up of three portions, one of which depends on the group only, another on the sub-group only and the third only on variation within the "cell." In our agricultural example this amounts to supposing that the yield is made up of three portions, one of which depends on the block only, the other on the manurial treatment only, and the third, which is supposed constant for all blocks and treatments, on other causes of variation.

Thus we have on this assumption

$$x_{uv} = t_u + b_v + \xi_{uv}$$

From which it easily follows that if  $s\bar{b} = \sum_{v=1}^s b_v$ ,  $r\bar{t} = \sum_{u=1}^r t_u$

$$\left. \begin{aligned} m_{u.} &= t_u + \bar{b} \\ m_{.v} &= \bar{t} + b_v \\ m &= \bar{b} + \bar{t} \\ m_{uv} &= t_u + b_v \end{aligned} \right\}$$

or 
$$m_{uv} - m_{u.} - m_{.v} + m = 0.$$

or 
$$ES(x_{uv} - \bar{x}_{u.} - \bar{x}_{.v} + \bar{x})^2 = (r-1)(s-1)\sigma^2 \quad (18)$$

Thus 
$$S(x_{uv} - \bar{x}_{u.} - \bar{x}_{.v} + \bar{x})^2 / (r-1)(s-1)$$

is an unbiased estimate of  $\sigma^2$ , our variance due to "error."\* Its distribution will be given by the previous theory.

The meaning of equation (14) now becomes clear. For we see that our unbiased estimate of  $\frac{S(m_{u.} - m)^2}{r-1}$

is 
$$\frac{S(\bar{x}_{u.} - \bar{x})^2}{r-1} - \frac{S(x_{uv} - \bar{x}_{u.} - \bar{x}_{.v} + \bar{x})^2}{(r-1)(s-1)} \quad (19)$$

In other words, if we again refer to our agricultural example, in order to estimate the variance due to treatments in our population, we must deduct from the item under "treatments" in our "mean square" column the item under "error." In the same way in order to estimate the variance due to blocks in our population we must deduct from the item under "blocks" in the "mean square" column the item under error.

\* This assumes that there is no significant interaction between treatments and blocks, which is the assumption usually made in a randomized block experiment. If there were a significant interaction it would be included in this estimate. The point could be tested by replicating the whole experiment.

The result may be put in an alternative form. Our estimate of

$$\sum_{u=1}^r (m_u - m)^2 / (r - 1)$$

will be

$$\frac{\sum_{u=1}^r (\bar{x}_u - \bar{x})^2}{r - 1} - \frac{\sum_{u=1, v=1}^{r, s} (x_{uv} - \bar{x}_u - \bar{x}_v + \bar{x})^2}{s(r - 1)(s - 1)} \quad (20)$$

Probably the left-hand side of (19) is a more satisfactory definition of "variance due to treatments" than the left-hand side of (20); however, both equations are equivalent, and the reader may take his choice.

The essential point to notice is that once the hypothesis that the sample is a random sample from a homogeneous population has been disproved, while our estimate of the variance due to error remains valid, the other items in the "mean square" column require a correction before they are unbiased estimates of the corresponding variances in the population.

I do not know whether this has been pointed out before. It is not perhaps a very important point in agricultural experiments, since an estimate of error is usually all that is required. But it becomes important as soon as we wish to compare the magnitudes of the various elements of variance.

It also brings out clearly that tests of significance must always be carefully distinguished from methods of estimation. A procedure which is adequate for the former is often inadequate for the latter.

There is one further point. If we wished to fit to our observed values  $x_{uv}$  a function of the form  $X_{uv} = t_u + b_v$  so as to make  $S(x_{uv} - X_{uv})^2$  a minimum, it can be easily shown that  $X_{uv} = \bar{x}_u + \bar{x}_v - \bar{x}$ ; so that our sum of squares due to error is, in fact, the sum of the squares of the residuals, when this method of fitting is adopted.

#### IV. *The Latin Square.*

(i) Since the Latin Square is not obviously included in the previous classification, it will be given special treatment here. As its use has so far been confined to agricultural experiments, agricultural language will from the outset be used.

Suppose we have  $r^2$  plots of land arranged in  $r$  rows and  $r$  columns,  $r$  different manurial treatments occurring on these plots in such a way that each treatment appears once in each column and each row.

Let  $X_{uv}$  be the yield of the plot in the  $u$ th column and  $v$ th row, let  $\bar{x}_u$  be the mean yield of column  $u$ ,  $\bar{x}_v$  the mean yield of row  $v$  and  $\bar{x}$  the mean yield of the plots receiving the same treatment as  $x_{uv}$ .



Let  $\bar{x}$  be the general mean.

Our fundamental identity here is

$$S(x_{uv} - \bar{x})^2 = S(\bar{x}_u. - \bar{x})^2 + S(\bar{x}_{.v} - \bar{x})^2 \\ + S(\bar{x}_t - \bar{x})^2 + S(x_{uv} - \bar{x}_u. - \bar{x}_{.v} - \bar{x}_t + 2\bar{x})^2. \quad (21)$$

$S$  being used as before *strictly* in the sense of summation over *every observation* in the sample. To prove this identity we remember that we have already seen that

$$S(x_{uv} - \bar{x})^2 = S(\bar{x}_u. - \bar{x})^2 + S(\bar{x}_{.v} - \bar{x})^2 + S(x_{uv} - \bar{x}_u. - \bar{x}_{.v} + \bar{x})^2$$

Here it is only necessary to prove that

$$S(x_{uv} - \bar{x}_u. - \bar{x}_{.v} - \bar{x}_t + 2\bar{x})^2 \\ = S(x_{uv} - \bar{x}_u. - \bar{x}_{.v} + \bar{x})^2 - S(\bar{x}_t - \bar{x})^2. \quad (22)$$

The left-hand side of this expression is equal to

$$S(x_{uv} - \bar{x}_u. - \bar{x}_{.v} + \bar{x})^2 + S(\bar{x} - \bar{x}_t)^2 \\ + 2S(\bar{x} - \bar{x}_t)(x_{uv} - \bar{x}_u. - \bar{x}_{.v} + \bar{x})$$

Considering the product term we have

$$S\bar{x}(x_{uv} - \bar{x}_u. - \bar{x}_{.v} + \bar{x}) = 0$$

To evaluate

$$S\bar{x}_t(x_{uv} - \bar{x}_u. - \bar{x}_{.v} + \bar{x})$$

let us sum first over all the plots which receive the  $t$ th treatment.

If these are  $x_{1a}, x_{2\beta}, x_{3\gamma}, \dots, x_{r\epsilon}$  we obtain

$$S\bar{x}_t(x_{uv} - \bar{x}_u. - \bar{x}_{.v} + \bar{x}) \\ = \Sigma \frac{1}{r} (x_{1a} + x_{2\beta} + \dots + x_{r\epsilon}) \\ \times \left\{ \begin{array}{l} x_{1a} - \bar{x}_1. - \bar{x}_{.a} + \bar{x} \\ + x_{2\beta} - \bar{x}_2. - \bar{x}_{.\beta} + \bar{x} \\ + x_{3\gamma} - \bar{x}_3. - \bar{x}_{.\gamma} + \bar{x} \\ + \dots \dots \dots \end{array} \right\}$$

$\Sigma$  denoting summation over all treatments.

This expression is equal to

$$\Sigma \frac{1}{r} (x_{1a} + x_{2\beta} + x_{3\gamma} + \dots)^2 \\ - \Sigma \frac{1}{r} (x_{1a} + x_{2\beta} + \dots)(\bar{x}_1. + \bar{x}_2. + \bar{x}_3. + \dots) \\ - \Sigma \frac{1}{r} (x_{1a} + x_{2\beta} + \dots)(\bar{x}_{.a} + \bar{x}_{.\beta} + \bar{x}_{.\gamma} + \dots) \\ + \Sigma (x_{1a} + x_{2\beta} + \dots)\bar{x}$$

But clearly

$$\begin{aligned}\bar{x}_1. + \bar{x}_2. + \bar{x}_3. + \dots &= r\bar{x} \\ \bar{x}_{.a} + \bar{x}_{.b} + \bar{x}_{.c} + \dots &= r\bar{x} \\ \Sigma \bar{x}(x_{1a} + x_{2b} + x_{3c} + \dots) &= r^2 \bar{x}^2\end{aligned}$$

Thus our expression is equivalent to

$$\begin{aligned}r\Sigma \bar{x}_i^2 - 2r^2 \bar{x}^2 + r^2 \bar{x}^2 \\ = r(\Sigma \bar{x}_i^2 - r\bar{x}^2) = S(\bar{x}_i - \bar{x})^2.\end{aligned}$$

Thus

$$\begin{aligned}S(x_{uv} - \bar{x}_u. - \bar{x}_{.v} - \bar{x}_t + 2\bar{x})^2 \\ = S(x_{uv} - \bar{x}_u. - \bar{x}_{.v} + x)^2 - S(\bar{x}_t - \bar{x})^2 \quad . \quad (23)\end{aligned}$$

and our identity is proved.

(ii) Let us first suppose we have a homogeneous normal population with variance  $\sigma^2$  and that we take a sample of  $r^2$  values and arrange these as in a Latin Square.

Then, as in the previous discussion, we shall easily find

$$\begin{aligned}ES(\bar{x}_u. - \bar{x})^2 &= (r-1)\sigma^2 \\ ES(\bar{x}_{.v} - \bar{x})^2 &= (r-1)\sigma^2 \\ ES(x_{uv} - \bar{x}_u. - \bar{x}_{.v} - \bar{x}_t + 2\bar{x})^2 &= (r-1)(r-2)\sigma^2\end{aligned}$$

It is easily shown that the multipliers of  $\sigma^2$  are in each case the corresponding degrees of freedom, and thus that each sum of squares divided by its corresponding degrees of freedom gives an estimate of variance whose distribution is given by the previous theory. In fact the analysis of variance is as follows :

			Sum of Squares.	Degrees of Freedom.
Columns	...	...	$S(\bar{x}_u. - \bar{x})^2$	$(r-1)$
Rows	...	...	$S(\bar{x}_{.v} - \bar{x})^2$	$(r-1)$
Treatments	...	...	$S(\bar{x}_t - \bar{x})^2$	$(r-1)$
Error	...	...	$S(x_{uv} - \bar{x}_u. - \bar{x}_{.v} - \bar{x}_t + 2\bar{x})^2$	$(r-1)(r-2)$
Total	...	...	$S(x_{uv} - \bar{x})^2$	$r^2 - 1$

Here our significance test consists in comparing by the "z" test the estimate of  $\sigma^2$  obtained from the "Treatment" item with that obtained from the "Error" item.

(iii) Once we have rejected the hypothesis that our sample is a random sample from a homogeneous normal population, we have, just as before, to make new assumptions about the nature of the population from which our sample is, in fact, drawn. The simplest way is to suppose—

(a) That the population consists of a set of sub-populations equal in number to the total number of different  $r$  by  $r$  Latin Squares.

(b) That each sub-population consists of  $r^2$  groups, identifiable by a column, row and treatment number, the position of treatments satisfying the Latin Square conditions.

(c) That the sample is formed by selecting a sub-population at random out of all the possible ones, and then selecting one individual, again at random, out of each group.

(d) That an individual yield consists of the sum of four portions due respectively to the column, row and treatment to which it belongs and to other sources of variation (error). It then follows that  $x_{uv} = m_{u.} + m_{.v} + m_t - 2m + \xi_{uv}$ , where the mean value of  $\xi_{uv}$  is zero for any one group.

Here  $m_{u.}$  is the population mean for the  $u$ th column,  $m_{.v}$  for the  $v$ th row,  $m_t$  for the  $t$ th treatment and  $m$  the general mean, and

$$m_{uv} = m_{u.} + m_{.v} + m_t - 2m.$$

We suppose  $\sigma^2$  to be the variance of the yields in any one group.

Then we can show that it is still true that

$$ES(x_{uv} - \bar{x}_{u.} - \bar{x}_{.v} - \bar{x}_t + 2\bar{x})^2 = (r-1)(r-2)\sigma^2$$

or that our estimate of error still holds and may therefore be used as a basis for calculating the standard errors of the treatment means.

Further,

$$\begin{aligned} ES(\bar{x}_t - \bar{x})^2 &= ES(m_t - m + \bar{\xi}_t - \bar{\xi})^2 \\ &= S(m_t - m)^2 + ES(\bar{\xi}_t - \bar{\xi})^2 \end{aligned}$$

But

$$\begin{aligned} E(\bar{\xi}_t - \bar{\xi})^2 &= E\bar{\xi}_t^2 - 2E\bar{\xi}_t\bar{\xi} + E\bar{\xi}^2 \\ &= \frac{\sigma^2}{r} - \frac{2\sigma^2}{r^2} + \frac{\sigma^2}{r^2} \\ &= \frac{\sigma^2}{r^2}(r-1) \end{aligned}$$

$$\begin{aligned} \text{Thus} \quad & ES(\bar{\xi}_t - \bar{\xi})^2 = \sigma^2(r-1) \\ \text{and} \quad & ES(\bar{x}_t - \bar{x})^2 = S(m_t - m)^2 + \sigma^2(r-1) \quad . \quad . \quad (24) \end{aligned}$$

Therefore our unbiased estimate of  $\frac{S(m_t - m)^2}{r-1}$  will be

$$\frac{S(\bar{x}_t - \bar{x})^2}{r-1} = \frac{S(x_{uv} - \bar{x}_{u.} - \bar{x}_{.v} - \bar{x}_t + 2\bar{x})^2}{(r-1)(r-2)}$$

or, if we prefer, we may say that our unbiased estimate of

$$\frac{\sum_{t=1}^r (m_t - m)^2}{r-1}$$

will be

$$\frac{\sum_{t=1}^r (\bar{x}_t - \bar{x})^2}{r-1} = \frac{\sum_{u=1}^r \sum_{v=1}^r (x_{uv} - \bar{x}_{u.} - \bar{x}_{.v} - \bar{x}_t + 2\bar{x})^2}{r(r-1)(r-2)} \quad . \quad (24 \text{ bis})$$

Thus, the item under "treatments" in the "mean square" column of the analysis is no longer our estimate of the variance due to

"treatments" in the population; an allowance for the error portion has first to be deducted. A similar allowance must be made in estimating the population variance due to columns and rows. Finally, we may remark that if an expression of the form  $X_{uv} = C_u + r_v + T_i$  is fitted by least squares to the data, our sum of squares due to error is, in fact,  $S(x_{uv} - X_{uv})^2$ , i.e. the sum of the squares of the residuals.

#### V. Conclusion.

The simpler cases of the analysis of variance have been dealt with. In particular it has been shown why it is correct to divide our "sums of squares" by the "degrees of freedom," and how it is that the additive property holds. That this is a perfectly general result has been indicated rather than proved in Section II (vi). A perfectly general proof that this must always hold good has been given by Fisher.<sup>(1)</sup> This proof puts in an algebraical form the geometrical arguments which he has so often used, and will be found of great interest by all serious students of the subject.

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XXI.—On the Dominance Ratio. By R. A. Fisher, M.A., Fellow of Gonville and Caius College. *Communicated by Professor J. ARTHUR THOMSON.*

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INTRODUCTION.

IN 1918, in a paper published in the *Transactions of the Royal Society of Edinburgh*, the author attempted an examination of the statistical effects in a mixed population of a large number of genetic factors, inheritance in which followed the Mendelian scheme. At that time, two misapprehensions were generally held with regard to this problem. In the first place, it was generally believed that the variety of the assumptions to be made about the individual factors—which allelomorph was dominant; to what extent did dominance occur; what were the relative magnitudes of the effects produced by the different factors; in what proportion did the allelomorphs occur in the general population, were the factors dimorphic or polymorphic; to what extent were they coupled,—besides the more general possibilities of preferential mating (homogamy), preferential survival (selection), and environmental effects, rendered it possible to reproduce any statistical resultant by a suitable specification of the population. It was, therefore, important to prove that when the factors are sufficiently numerous, the most general assumptions as to their individual peculiarities lead to the same statistical results. Although innumerable constants enter into the analysis, the constants necessary to specify the statistical aggregate are relatively few. The total variance of the population in any feature is made up of the elements of variance contributed by the individual factors, increased in a calculable proportion by the effects of homogamy in associating together allelomorphs of like effect. The degree of this association, together with a quantity which we termed the Dominance Ratio, enter into the calculation of the correlation coefficients between husband and wife, and between blood relations. Special causes, such as epistacy, may produce departures, which may in general be expected to be very small from the general simplicity of the results; the whole investigation may be compared to the analytical treatment of the Theory of Gases, in which it is possible to make the most varied assumptions as to the accidental circumstances, and even the essential nature of

the individual molecules, and yet to develop the general laws as to the behaviour of gases, leaving but a few fundamental constants to be determined by experiment.

In the second place, it was widely believed that the results of biometrical investigation ran counter to the general acceptance of the Mendelian scheme of inheritance. This belief was largely due to the narrowly restricted assumptions as to the Mendelian factors, made by Pearson in his paper of 1903 (6). It was there assumed that the factors were all equally important, that the allelomorphs of each occurred in equal numbers, and that all the dominant genes had a like effect. The effect of homogamy was also left out of consideration, and it is to this that must be ascribed the much lower correlations given by calculation, compared to those actually obtained. When the more general system came to be investigated, it was found to show a surprisingly complete agreement with the experimental values, and to indicate with an accuracy which could not otherwise be attained, how great a proportion of the variance of these human measurements is to be ascribed to heritable factors.

At the time when the paper of 1918 was written, it was necessary, therefore, to show that the assumption of multiple, or cumulative, factors afforded a working hypothesis for the inheritance of such apparently continuous variates as human stature. This view is now far more widely accepted: Mendelian research has with increasing frequency encountered characters which are evidently affected by many separate factors. In some fortunate circumstances, as in *Drosophila*, it has been possible to isolate and identify the more important of these factors by experimental breeding on the Mendelian method; more frequently, however, and especially in the case of the economically valuable characters of animals and plants, no such analysis has been achieved. In these cases we can confidently fall back upon statistical methods, and recognise that if a complete analysis is unattainable it is also unnecessary to practical progress.

This fact is meeting with increasing recognition in the United States, and a considerable number of mathematical investigations have been published dealing with the statistical effects of various systems of mating (Wentworth and Remick, 1916; Jennings, 1916, 1917; Robbins, 1917, 1918). A number of the simpler results of my 1918 paper have since been confirmed by independent American investigators (Wright, 1921). The present note is designed to discuss the distribution of the frequency ratio of the allelomorphs of dimorphic factors, and the conditions under which the variance of the population may be maintained. A number of points of general interest are shown to flow from purely statistical premises.

Recent work in genetics (East and Jones, 1920) leads unavoidably to the conclusion that inbreeding is not harmful in itself, but is liable to appear harmful only through the emergence of harmful recessive characters. This raises the question as to why recessive factors should tend to be harmful, or why harmful factors should tend to be recessive: unless this association exist we should expect to obtain great improvements by inbreeding ordinarily crossbred species, as often as great deterioration. The statistical reason for this association is clear from the distribution of the ratio of allelomorph frequency which occurs under genotypic selection, for, if we assume that adaptation is the result of selection, the majority of large mutations must be harmful, and these can only be incorporated in the common stock in the sheltered region where the rare recessives accumulate (fig. 4).<sup>\*</sup> Similarly there are many well-attested cases of the crossbred (heterozygous) individual showing surprising vigour; but it is not obvious that there is any biological reason for the heterozygote to be more vigorous than the two homozygotes. From a consideration of the stability of the frequency ratios, however, it appears that there will only be stable equilibrium if the heterozygote is favoured by selection against both the homozygotes: naturally this will occur only in a minority of factors, but when it occurs such a factor will be conserved. In the opposite case it will certainly be eliminated.

Cases in which the heterozygote is favoured by selection in preference to both homozygous forms have an additional interest in that these cases, when the selection is intense, may form the basis upon which is built up a system of balanced lethal factors. Muller (1918) has shown that such systems will tend to be built up when selection strongly favours the heterozygote, and has explained how in the light of such systems the majority of the phenomena, including the "mutations," of *Oenothera*, find a genetic explanation.

The interesting speculation has recently been put forward that random survival is a more important factor in limiting the variability of species than preferential survival (Hagedoorn, 4). The ensuing investigation negatives this suggestion. The decay in the variance of a species breeding at random without selection, and without mutation, is almost inconceivably slow: a moderate supply of fresh mutations will be sufficient to maintain the variability. When selection is at work even to the most trifling extent, the new mutations must be much more numerous in order to

<sup>\*</sup> On the Lamarckian theory of evolution, on the other hand, where most, or all, mutations are assumed to be beneficial, we should expect by inbreeding, which uncovers the accumulated mutations in this region, to make great and immediate progress.

maintain equilibrium. That such is the actual state of the case in mankind may be inferred from the fact that the frequency distribution of the numerical proportion of the allelomorphs, calculated on the assumption of selection maintained in equilibrium by occasional mutation, leads to the value of the Dominance Ratio which is actually observed. In all cases it is worth noting that the rate of mutation required varies as the variance of the species, but diminishes as the number of individuals is increased. Thus a numerous species, with the same frequency of mutation, will maintain a higher variability than will a less numerous species: in connection with this fact we cannot fail to remember the dictum of Charles Darwin, that "wide ranging, much diffused and common species vary most" (1, chap. ii).

#### 1. EQUILIBRIUM UNDER SELECTION.

Let the three phases of a dimorphic factor be born in any generation in the proportion

$$P : 2Q : R,$$

then the proportion of the two allelomorphic genes will be

$$P + Q : Q + R, \quad \text{or} \quad p : q;$$

if by selection those that become parents are in the proportion

$$aP : 2bQ : cR, \quad \text{where} \quad aP + 2bQ + cR = 1,$$

then the proportion born in the next generation will be

$$(aP + bQ)^2 : 2(aP + bQ)(bQ + cR) : (bQ + cR)^2;$$

equilibrium is thus only possible if  $Q^2 = PR$ , *i.e.*  $P = p^2$ ,  $Q = pq$ ,  $R = q^2$ , and if  $aP + bQ = p$ ,  $bQ + cR = q$ .

Hence it follows that, if

$$a = 1 + \alpha, \quad b = 1 + \beta, \quad c = 1 + \gamma,$$

$$\frac{\alpha}{p^2} = -\frac{\beta}{pq} = \frac{\gamma}{q^2}$$

specifies the condition of equilibrium.

If selection favours the homozygotes, no stable equilibrium will be possible, and selection will then tend to eliminate whichever gene is below its equilibrium proportion; such factors will therefore not commonly be found in nature: if, on the other hand, the selection favours the heterozygote, there is a condition of stable equilibrium, and the factor will continue in the stock. Such factors should therefore be commonly found, and may explain instances of heterozygote vigour, and to some extent the deleterious effects sometimes brought about by inbreeding.



If the selective action is sufficiently powerful, it may lead in these cases to the establishment of a balanced lethal system.

## 2. THE SURVIVAL OF INDIVIDUAL GENES.

If we consider the survival of an individual gene in such an organism as an annual plant, we may suppose that the chance of it appearing in the next generation in 0, 1, 2, 3 individuals to be

$$p_0, p_1, p_2, \dots$$

where

$$p_0 + p_1 + p_2 + \dots = 1.$$

If

$$f(x) = p_0 + p_1x + p_2x^2 + \dots$$

then evidently if there were two such genes in the first generation, the chance of occurrence in  $r$  individuals, or more strictly, in  $r$  homologous loci, in the second generation, will be the coefficient of  $x^r$  in

$$(f(x))^2.$$

It follows that the chance of a single gene occurring in  $r$  homologous loci, in the third generation, will be coefficient of  $x^r$  in

$$f(f(x)).$$

The form of  $f(x)$  will vary from species to species, and in the same species according to the stage of development on which we fix our attention. For simplicity we shall suppose that the successive generations are enumerated at the same stage of development. For the purpose of an evolutionary argument it is indifferent at what stage of development the enumeration is made: in general it will be most convenient to fix our attention on that stage at which the species is least numerous.

In certain important cases the form of  $f(x)$  may be calculated. In a field of cross-fertilised grain each mature and ripened plant is the mother of a considerable number of grains, and the father, possibly, of an almost unlimited number. If the number of the species is nearly constant, the average number of its progeny which are destined to become mature is very nearly 2. Or since each gene of a homologous pair occurs in half the gametes, the average number of mature plants in the second generation in which it occurs is 1. Each ovule, therefore, or each pollen grain has individually a very small chance of surviving, and the proportions  $p_0, p_1, p_2$ , will be closely given by the Poisson series

$$e^{-1} \left( 1, 1, \frac{1}{2!}, \frac{1}{3!}, \dots \right)$$

In the more general case in which the number of the species is not stationary but increases in each generation in the ratio  $m : 1$ ,  $m$  being near to unity, the series will be

$$e^{-m} \left( l, m, \frac{m^2}{2!}, \frac{m^3}{3!}, \dots \right)$$

and  $f(x) = e^{m(x-1)}$ . The chance of extinction of a single gene in one generation is  $e^{-m}$ , where  $m$  is near to unity. In other species in which an individual may survive for many breeding seasons, or in which the generation is of indeterminate length, the form of the function  $f(x)$  will be modified: it is sufficiently clear, however, that if we consider that stage in an animal's or plant's life-history at which reproduction is about to commence, the form of the function will not be very different, and the chance of extinction of a particular gene, thus far established in the species, will be

$$e^{-l},$$

where  $l$  is a small number not greatly different from unity.\* The arbitrary element thus introduced into the question of the survival of a mutant gene is due to the fact that in the first place its survival depends on that of the individual in which it occurs, and this chance is variable from species to species; once, however, it has reached the point of existing in an adult individual capable of leaving many offspring, the conditions of its survival are closely similar in all cases. While it is rare, its survival will be at the mercy of chance, even if it is well fitted to survive. Using the above expression,

$$f(x) = e^{x-1},$$

it may be seen that only about 2 per cent. will survive 100 generations, while those that do will on the average be represented in some 50 individuals. Only when the number of individuals affected becomes large will the effect of selection predominate over that of random survival, though even then only a very small minority of the population may be affected.

### 3. FACTORS NOT ACTED ON BY SELECTION.

If  $p$  be the proportion of any gene, and  $q$  of its allelomorph in a dimorphic factor, then in  $n$  individuals of any generation we have  $2np$  genes scattered at random. Let

$$\cos \theta = 1 - 2p$$

where  $\theta$  lies between 0 and  $\pi$ .

\* An upper limit can be set to  $l$  by the mere fact of segregation, for in the case of the most uniform possible reproduction, when each individual bears 2 offspring the chance of extinction of any gene is  $\frac{1}{2}$ , so that  $l$  cannot exceed 1.4.

Further, if a second generation of  $n$  individuals be now formed at random, the standard departure of  $p$  from its previous value will be

$$\sigma_p = \sqrt{\frac{pq}{2n}},$$

hence,

$$\sigma_\theta = \sqrt{\frac{pq}{2n}} \frac{d\theta}{dp} = \frac{1}{\sqrt{2n}}.$$

The fact that this is independent of  $\theta$  makes it easy to calculate the changes in the distribution of  $\theta$ , in the absence of selection, for let  $y(\theta) d\theta$  represent the distribution of  $\theta$  in any one generation, the distribution in the next will be given by

$$\begin{aligned} y + \Delta y &= \int_0^\pi \frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{\delta\theta^2}{2\sigma^2}} \left( y + y'\delta\theta + \frac{\delta^2\theta^2}{2!}y'' + \dots \right) \\ &= y + \frac{\sigma^2}{2}y'' + \dots \end{aligned}$$

Now  $\sigma^2$  is very small, being  $\frac{1}{2n}$ , so that measuring time in generations, we have

$$\frac{\partial y}{\partial T} = \frac{1}{4n} \frac{\partial^2 y}{\partial \theta^2}.$$

Since we have drawn no distinction between the gene and its allelomorph, we are only concerned with symmetrical solutions: the stationary case is

$$y = \frac{A}{\pi},$$

where  $A$  is the number of factors present.

Besides this, we have when  $y$  is increasing

$$y = A_0 e^{kT} \frac{p}{2 \sinh \frac{1}{2} p\pi} \cdot \cosh p\left(\theta - \frac{\pi}{2}\right),$$

and when  $y$  is decreasing

$$y = A_0 e^{-kT} \frac{p}{2 \sin \frac{1}{2} p\pi} \cdot \cos p\left(\theta - \frac{\pi}{2}\right),$$

for which

$$k = \frac{p^2}{4n}.$$

#### 4. TERMINAL CONDITIONS.

If we represent by  $e^{-t}$  the chance that a particular gene borne by a single individual will not be represented in the next generation, the chance of extinction for a factor of which  $b$  genes are in existence will be

$$e^{-bt}.$$

When  $\theta$  is near to 0,  $p$  which is always equal to  $\sin^2 \frac{\theta}{2}$ , will be very nearly equal to  $\frac{1}{4}\theta^2$ . Let

$$t = \sin \frac{1}{2}\theta,$$

then the number of genes in existence is  $2nt^2$ , and the chance of their extinction in one generation is  $e^{-2nt^2}$ .

This chance is negligible save when  $t$  is very small, and may be equated to  $\frac{1}{2}\theta$ ; hence the number of genes exterminated in any one generation

$$\begin{aligned} & 2 \int_0^1 ye^{-2nt^2} d\theta \\ &= 4 \int_0^1 ye^{-2nt^2} dt. \end{aligned}$$

In the stationary case  $y = \frac{A}{\pi}$ , and the number of genes exterminated will be

$$\frac{A}{\pi} \cdot \frac{2\sqrt{2\pi}}{\sqrt{4ln}} = A\sqrt{\frac{2}{\pi ln}},$$

if new mutations occur at a rate  $n\mu$ , then this equilibrium will be possible if

$$A = \sqrt{\frac{\pi l}{2}} n^{\frac{1}{2}} \mu.$$

For species in this stationary state the variance will vary (1) as the rate of mutation, (2) as the number of the population raised to the power of  $\frac{3}{2}$ , (3) as  $\sqrt{l}$ , a quantity which will seldom differ much from unity. Using the variate  $z = \log_e \frac{p}{q}$ , the distribution for this case is shown in fig. 1.

## 5. THE HAGEDOORN EFFECT.

In the absence of mutation, extinction will still go on, and the number of factors must diminish, hence we may put for this case

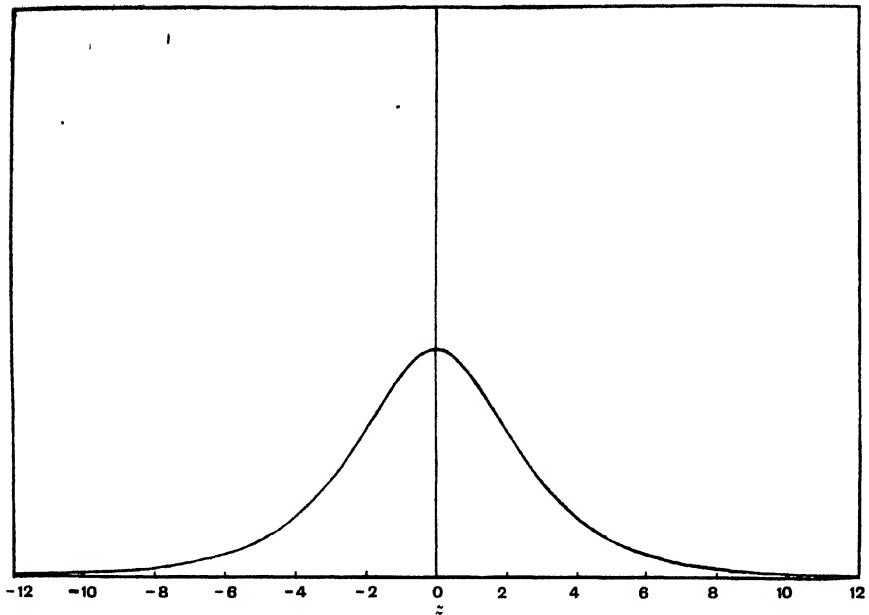
$$y = A_0 e^{-kt} \cdot \frac{p}{2 \sin \frac{1}{2}p\pi} \cdot \cos p\left(\theta - \frac{\pi}{2}\right).$$

If  $\theta$  is small,

$$\begin{aligned} \cos p\left(\theta - \frac{\pi}{2}\right) &= \cos \frac{1}{2}p\pi + p\theta \sin \frac{1}{2}p\pi - \frac{1}{2}p^2\theta^2 \cos \frac{1}{2}p\pi \dots \\ &= \cos \frac{1}{2}p\pi + 2p \sin \frac{1}{2}p\pi \cdot t - 2p^2 \cos \frac{1}{2}p\pi \cdot t^2 \dots, \end{aligned}$$

so that the rate of extinction is

$$A_0 e^{-kt} \frac{p}{2 \sin \frac{1}{2}p\pi} \cdot \sqrt{\frac{2\pi}{ln}} \left\{ \cos \frac{1}{2}p\pi + 2p \sin \frac{1}{2}p\pi \cdot \sqrt{\frac{2}{4 ln}} \right\}$$



Distribution of the logarithmic frequency ratio  $\left(z = \log \frac{p}{q}\right)$  of the allelomorphs of a dimorphic factor.

FIG. 1.— $df = \frac{1}{2\pi} \text{sech}^2 \frac{1}{2} z dz$ ;

represents the distribution when, in the absence of selection, fortuitous extinction is counterbalanced by mutation. Dominance Ratio = '2308.

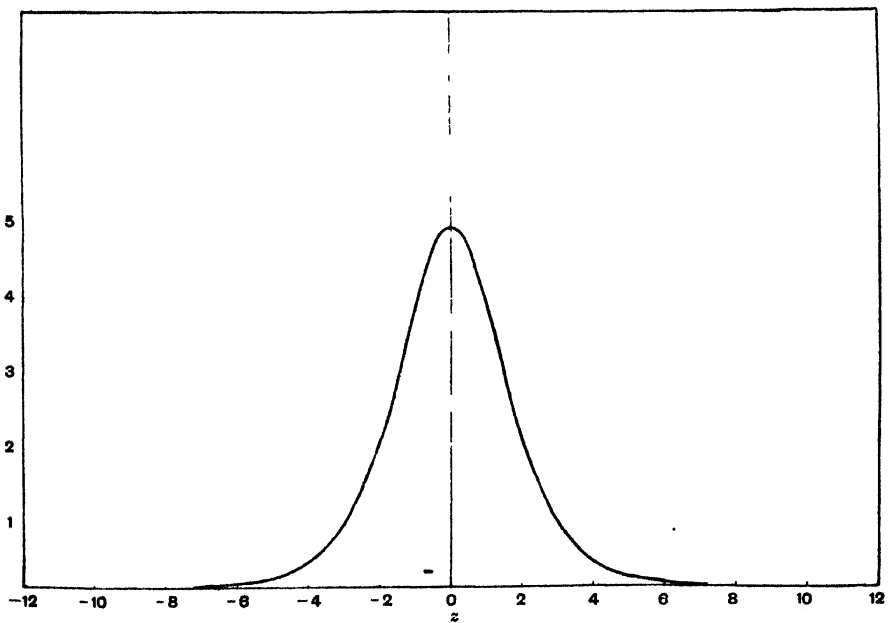


FIG. 2.— $df = \frac{1}{2} \text{sech}^2 \frac{1}{2} z dz$ ;

represents the distribution when, in the absence of selection and mutation, the variance is steadily decaying owing to fortuitous extinction of genes. Dominance Ratio = '2500. This is the condition emphasised by Hagedoorn.

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the third term being evidently negligible compared to the first. For  
equilibrium, therefore,

$$k = p\sqrt{\frac{2\pi}{ln}} \left\{ \frac{1}{2} \cot \frac{1}{2}p\pi + \frac{p}{2\pi ln} \right\}.$$

Remembering that  $k = \frac{p^2}{4n}$ , we have

$$\frac{p^2}{n} \left( \frac{1}{2} - \frac{1}{l} \right) = \sqrt{\frac{2\pi}{ln}} \frac{p}{2} \cot \frac{1}{2}p\pi.$$

Hence  $\cot \frac{1}{2}p\pi$  is of the order  $\frac{1}{\sqrt{n}}$  and is very small, so that  $p$  is near to 1.

Then

$$k = \frac{1}{4n}.$$

This is a very slow rate of diminution, a population of  $n$  individuals breeding at random would require  $4n$  generations to reduce its variance in the ratio 1 to  $e$ , or  $2.8 n$  generations to halve it. As few specific groups contain less than 10,000 individuals between whom interbreeding takes place, the period required for the action of the Hagedoorn effect, in the entire absence of mutation, is immense. It will be noticed that since  $l$  is always less than 1.4 in species stationary in number, the solution above makes  $p$  slightly greater than 1, which strictly would indicate negative frequencies at the extremes: the value of  $k$  is, however, connected with the curvature in the central portion of the curve, and the small distortion at the extremes, where the assumptions, upon which our differential equation is based, break down, will not affect its value. (Fig. 2 shows the distribution of  $z = \log \frac{p}{q}$ .)

The number by which the number of factors current is reduced in each generation is  $\frac{A}{4n}$ , and since this number depends on the general form of the distribution curve, it will not be diminished by a number of mutations of the same order. The effect of such very rare mutations would merely be to adjust the terminal of the curve until the rate of extinction is increased sufficiently to counterbalance the additional mutations. It is probable, however, that  $\mu$  is always far greater than is necessary to make this state of affairs impossible, save in the case of a small colony recently isolated from a very variable species. In this case, with  $n$  small and  $A$  large,  $\mu$  might for a time be of the order  $An^{-2}$ , rather than of the order  $An^{-1}$ , or  $An^{-1}$ .

In the case of a population with  $A$  factors, with a supply of fresh

mutations sufficient only to be in equilibrium with a smaller number  $B$  factors, we may put

$$B\sqrt{\frac{2}{\pi \ln}} = \frac{Ap}{2 \sin \frac{1}{2}p\pi} \cdot \cos \frac{1}{2}p\pi \cdot \sqrt{\frac{2\pi}{\ln}},$$

or,

$$\frac{B}{A} = \frac{1}{2}p\pi \cot \frac{1}{2}p\pi,$$

so that if

$$\frac{a}{\tanh a} = \frac{B}{A}, \quad 0 < a < \frac{\pi}{2},$$

$$p = \frac{2a}{\pi},$$

and

$$k = \frac{a^2}{\pi n}.$$

Similarly, if  $B > A$ , the rate of increase in variance may be calculated from the equations

$$\frac{a}{\tanh a} = \frac{B}{A},$$

$$k = \frac{a^2}{\pi n}.$$

The rate of decrease, therefore, cannot, in the absence of selection, exceed the value indicated by  $k = \frac{1}{4n}$ ; no such limit can be assigned to the rate of increase.

## 6. UNIFORM GENETIC SELECTION.

In section 1 we have seen that the effects of selection on any Mendelian factor may be expressed by the triple ratio  $a : b : c$  representing the relative fitness of the three phases. Only when  $b$  exceeds both  $a$  and  $c$  is there a condition of stable equilibrium; when  $b$  is less than both  $a$  and  $c$  there is a condition of unstable equilibrium; and such factors will tend rapidly to disappear from the stock. Generally, however, we may expect that either  $b$  will be intermediate, or equal to  $a$ , the value for the dominant homozygote. Two hypothetical cases may, therefore, be considered. (1), in which  $b$  is the geometric mean of  $a$  and  $c$ , and the selection merely affects the proportion of the allelomorphic genes; we may call this uniform genetic selection; and (2), in which  $b$  is equal to  $a$ , which we may call uniform genotypic selection.

In uniform genetic selection the genetic ratio will be altered in a constant ratio  $r$  in each generation, so that after  $n$  generations of selection we have

$$\frac{p}{q} = r^n \frac{p_0}{q_0},$$

evidently  $r = \frac{a}{b} = \frac{b}{c}$  of section 1.

We may suppose that usually  $r$  is near to unity, and  $\log r$ , which may be positive or negative, may be considered to be of the order of 1 per cent. Let  $\log r = a$ , then for different factors  $a$  will have different values, indifferently positive and negative, since we have no reason to suppose that the selection favours either dominant or recessive characters. The mean square value of  $a$  for different factors we shall write  $\sigma_a^2$ .

For any factor

$$\frac{d}{dT} \log \frac{p}{q} = a;$$

therefore

$$\frac{dp}{dT} = pq a,$$

$$\frac{d\theta}{dT} = a \sqrt{pq}.$$

The factors which in one generation are at  $\theta$ , will in the next be scattered owing to two causes: (1) random survival causing variance,  $\frac{1}{2n}$ ; (2) selection causing variance,  $pq \sigma_a^2 (= \frac{1}{4} \sin^2 \theta \cdot \sigma_a^2)$ . The total variance at any point will be

$$\frac{1}{2n} + \frac{1}{4} \sigma_a^2 \sin^2 \theta,$$

and so long as  $\sigma_a^2$  is small as we have supposed, the equilibrium distribution will be

$$y \propto \frac{1}{\sqrt{\sin^2 \theta + \frac{2}{n \sigma_a^2}}},$$

or nearly

$$y = \frac{A}{2 \log (\sigma_a \sqrt{8n})} \cdot \frac{1}{\sqrt{\sin^2 \theta + \frac{2}{n \sigma_a^2}}}.$$

$n$  being large compared with  $\frac{1}{\sigma_a^2}$ , the effects of selection are, for the more important factors, much more influential than those of random survival. At the extremes, however, for very unequally divided factors the latter is the more important cause of variation. (The distribution of  $z = \log \frac{p}{q}$  is shown in fig. 3.)

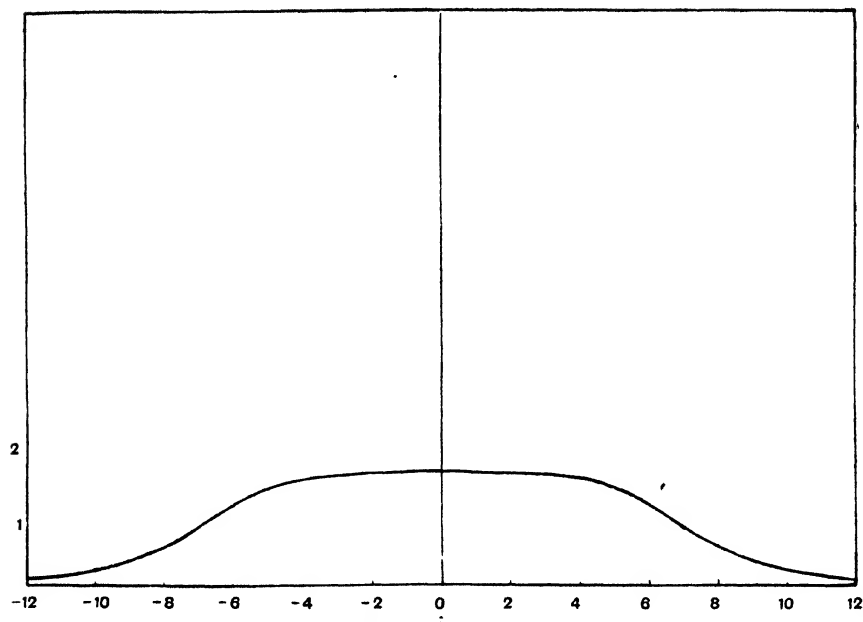
The amount of mutation needed to maintain the variability with this amount of selection may be calculated from the terminal ordinate

$$\frac{A \sigma_a \sqrt{\frac{n}{2}}}{2 \log (\sigma_a \sqrt{8n})},$$

whence

$$n\mu = \sqrt{\frac{2\pi}{ln}} \cdot \frac{A \sigma_a \sqrt{\frac{n}{2}}}{2 \log (\sigma_a \sqrt{8n})} = \frac{A \sigma_a \sqrt{\frac{\pi}{l}}}{2 \log (\sigma_a \sqrt{8n})}.$$

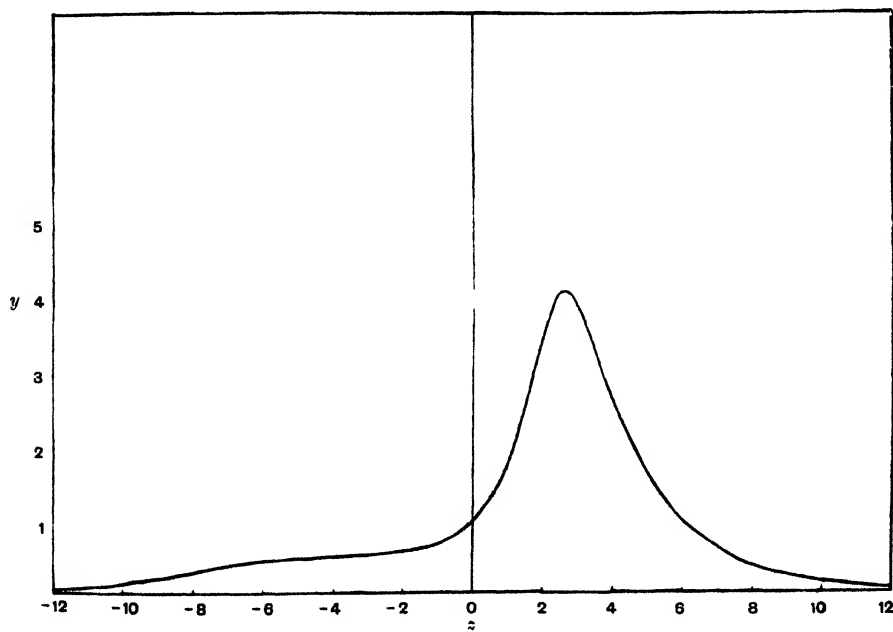




Distribution of the logarithmic frequency ratio.

$$\text{FIG. 3.} -df \propto \frac{dz}{\sqrt{1+k^2 \cosh^2 \frac{1}{2}z}}; k=1;$$

genetic selection counterbalanced by mutation. Dominance Ratio, '2000.



$$\text{FIG. 4.} -df \propto \frac{dz}{\sqrt{e^{-z} \operatorname{sech}^2 \frac{1}{2}z + k^2 \cosh^2 \frac{1}{2}z}}; k=1;$$

genotypic selection, with complete dominance, counterbalanced by mutation. Dominance Ratio, '3333. This is the probable condition of natural species, including man. Note the accumulation of rare recessives.

Since the logarithm does not increase very rapidly, we may say approximately that  $A$  is proportional to  $\frac{n\mu}{\sigma_a}$ .

It will be seen that to maintain the same amount of variability, as in the case of equilibrium in the absence of selection (section 4), the rate of mutation must be increased by a factor of the order  $\sigma_a \sqrt{n}$ . Even in the low estimate we have made of the intensity of selection on the majority of factors, this quantity will usually be considerable. The existence of even the slightest selection is in large populations of more influence in keeping variability in check than random survival.

A further effect of selection is to remove preferentially those factors for which  $a$  is high, and to leave a predominating number in which  $a$  is low. In any factor  $a$  may be low for one of two reasons: (1) the effect of the factor on development may be very slight, or (2) the factor may effect changes of little adaptive importance. It is therefore to be expected that the large and easily recognised factors in natural organisms will be of little adaptive importance, and that the factors affecting important adaptations will be individually of very slight effect. We should thus expect that variation in organs of adaptive importance should be due to numerous factors, which individually are difficult to detect.

Owing to this preferential removal of important factors the above solution only truly represents an equilibrium of the variability of the species under absolutely uniform conditions of selection when the new mutations which arise have the same frequency distribution of relative importance as those removed by selection. It must be remembered, however, that the change of variability even by selection is a very slow process, and that gradual changes in the physical and biological environment of a species will alter the values of  $a$  for each factor, so tending to neutralise the tendency of selection to lower the value of  $\sigma_a$ . Nevertheless,  $a$  will be on the whole numerically smaller for factors in the current stock than it is for fresh mutations.

## 7. UNIFORM GENOTYPIC SELECTION.

If the heterozygote is selected to the same extent as the dominant, or  $b=a$ , it is easy to see by writing down the first generation, that a genetic ratio  $p:q$ , becomes in one generation by selection  $\frac{p'}{q} \frac{a}{ap+cq}$ ; or, writing  $1+\beta$  for  $\frac{a}{c}$ ,

$$\frac{p}{q} \frac{1+\beta}{1+p\beta};$$

or, when  $\beta$  is small,

$$\frac{p}{q}(1 + q\beta).$$

Such selection is therefore equivalent to a genetic selection

$$a = q\beta.$$

Now

$$\frac{d\theta}{dT} = a \sqrt{pq} = \beta q \sqrt{pq},$$

and for the variance caused by selection, instead of  $pq\sigma_a^2$ , as in Section 6, we now write  $pq^3\sigma_\beta^2$ : we have then for the total variance produced in one generation in the value of  $\theta$ ,

$$\begin{aligned} & \frac{1}{2n} + \frac{1}{16} \sin^2 \theta (1 + \cos \theta)^2 \sigma_\beta^2 \\ &= \frac{1}{2n} + \sin^2 \frac{1}{2} \theta \cos^6 \frac{1}{2} \theta \cdot \sigma_\beta^2, \end{aligned}$$

and the equilibrium distribution will be

$$y \propto \frac{1}{\sqrt{\sin^2 \frac{1}{2} \theta \cos^6 \frac{1}{2} \theta + \frac{1}{2n\sigma_\beta^2}}}.$$

It is important to notice that this distribution, unlike those hitherto considered, is unsymmetrical, factors of which the dominant phase is in excess are in the majority. This has an important influence on the value of the dominance ratio.

If  $2n\sigma_\beta^2$  is large, we can write with sufficient accuracy \*

$$y = \frac{A}{1.4022(2n\sigma_\beta^2)^{\frac{1}{2}} + \frac{2}{3} \log(8n\sigma_\beta^2) - \frac{2}{3}} \cdot \frac{1}{\sqrt{\sin^2 \frac{1}{2} \theta \cos^6 \frac{1}{2} \theta + \frac{1}{2n\sigma_\beta^2}}}.$$

The terminal ordinate therefore varies nearly as  $(2n\sigma_\beta^2)^{\frac{1}{2}}$ , and for large populations in equilibrium,  $\mu$  varies as  $n^{-\frac{1}{2}}$  and as  $\sigma_\beta^{\frac{1}{2}}$ .

Genotypic selection resembles genetic selection in diminishing the amount of variability which a given frequency of mutation can maintain, or *per contra*, increasing the amount of mutation needed to maintain a given amount of variability; it differs, however, in being comparatively inactive in respect of factors in which the dominant allelomorph is in excess, and consequently in allowing a far greater number of factors to exist in this region (see fig. 4).

\* I am indebted to Mr E. Gallop, Gonville and Caius College, Cambridge, for the value of the definite integral. Mr Gallop has shown that the three terms given are the heads of three series in descending powers of  $n\sigma_\beta^2$ , in which the integral may be expanded.

Now when dominance is complete, the dominance ratio from a group of factors having the same ratio  $\frac{p}{q}$  is

$$= \frac{1}{1 + 2\frac{q}{p}},$$

for in the notation of our previous paper

$$\delta^2 = 4p^2q^2a^2,$$

and

$$a^2 = 4p^2q^2\alpha^2\left(1 + 2\frac{q}{p}\right),$$

where  $a$  is half the difference between the two homozygous forms (3, p. 404).

The dominance ratio is therefore raised by an excess of factors in which the dominant gene is the more numerous, such as occurs under genotypic selection.

## 8. THE DOMINANCE RATIO.

The distribution found for the ratio  $\frac{p}{q}$  or for the value of  $\theta$ , which indicates the same quantity, in sections 3 to 7, enable us to calculate the value attained by the dominance ratio under each of the suppositions there considered.

1. In the Hagedoorn condition, where the variance is steadily decaying by random survival, in the absence of mutations or selection,

$$df = \frac{1}{2}A \sin \theta d\theta,$$

writing  $\phi = \frac{1}{2}\theta$ , then  $p = \sin^2 \phi$ ,  $q = \cos^2 \phi$ ,

whence

$$\epsilon^2 = S(\delta^2) = 8A\overline{a^2} \int_0^{\frac{1}{2}\pi} \sin^5 \phi \cos^5 \phi d\phi,$$

$$\sigma^2 = S(a^2) = 8A\overline{a^2} \int_0^{\frac{1}{2}\pi} (\sin^5 \phi \cos^5 \phi + 2 \sin^3 \phi \cos^7 \phi) d\phi,$$

and

$$\frac{\epsilon^2}{\sigma^2} = \frac{1}{1 + 2 \cdot \frac{3}{2}} = .2500.$$

2. When in the absence of selection, sufficient mutations take place to counteract the effect of random survival

$$df = \frac{2A}{\pi} d\phi,$$

and we have to consider the ratio of the integrals

$$\int_0^{\frac{1}{2}\pi} \sin^4 \phi \cos^4 \phi d\phi, \quad \int_0^{\frac{1}{2}\pi} \sin^2 \phi \cos^6 \phi d\phi,$$

which are in the ratio 3 : 5.

The dominance ratio is therefore

$$\frac{3}{3+2(5)} = \cdot 2308;$$

the greater variation in the ratio  $\frac{p}{q}$  showing itself in a lower dominance ratio.

3. In the third symmetrical case, when genetic selection is at work, the variation of  $\frac{p}{q}$  is even greater (fig. 3); since both  $\sigma^2$  and  $\alpha^2$  contain the factor  $p^2q^2$ , the factors in which  $p$  or  $q$  is very small, make no appreciable contribution to these quantities, consequently we only consider the central portion of the distribution, where

$$df \propto \frac{d\phi}{\sin \phi \cos \phi},$$

the intensity of selection appearing only as a constant factor, and therefore influencing the range of variation of the species, but not its dominance ratio. Here we have the integrals

$$\int_0^{\frac{1}{2}\pi} \sin^3 \phi \cos^3 \phi d\phi \quad \text{and} \quad \int_0^{\frac{1}{2}\pi} \sin \phi \cos^5 \phi d\phi,$$

leading to a dominance ratio

$$\frac{1}{1+4} = \cdot 2000.$$

4. In the case of genotypic selection, which case most nearly reproduces natural conditions, the distribution in the centre of the range is

$$df \propto \frac{d\phi}{\sin \phi \cos^3 \phi},$$

consequently the two integrals with which we are concerned

$$\int_0^{\frac{1}{2}\pi} \sin^3 \phi \cos \phi d\phi, \quad \int_0^{\frac{1}{2}\pi} \sin \phi \cos^3 \phi d\phi$$

are now equal, and the dominance ratio is raised to  $\frac{1}{3}$ .

In considering the interpretation of the dominance ratio, in our previous inquiry, we found that for symmetrical distribution the value  $\frac{1}{3}$  occurred as a limiting value when the standard deviation of  $z \left( = \log \frac{p}{q} \right)$  was made zero. Since the dominance ratio calculated from observed human correlations averaged  $\cdot 32$ , with a standard error about  $\cdot 03$ , we were led to consider that either the allelomorphs concerned occurred usually in nearly equal numbers, a supposition for which we saw no

rational explanation, or that the value of the dominance ratio had been raised by the prevalence of epistacy (non-linear interaction of factors), a suggestion for which no direct evidence could be adduced.

In the light of the above discussion in which we have deduced the distribution of allelomorphic ratios from the conditions of equilibrium with selective influences, from which condition it is probable that natural species do not widely depart, we find that the value  $\frac{1}{2}$  for the dominance ratio is produced by the asymmetry of the distribution, and in such a manner as to be independent of the activity of the selective agencies, provided that this exceeds a certain very low level. When differential survival to the extent of only about 1 per cent. in a generation affects the different Mendelian factors, in a population of only a million, and far more for more powerful selection, or a larger population, the dominance ratio will be very close to its characteristic value of  $\frac{1}{2}$ .

The importance of the fact that this ratio is independent of the intensity of selection, lies not only in the fact that the intensity of selection is usually incapable of numerical estimation, but in the fact that factors having effects of different magnitudes on the soma, which are therefore exposed to selection of varying intensity, and contribute very different quota to the variance, are all affected in the same manner; those factors which by their insignificance might be exposed to selective influences which are not large compared to the effects of random survival will be precisely those which have little weight in computing the dominance ratio.

#### 9. ASSORTATIVE MATING.

With assortative mating it has been shown (3, p. 414) that the deviations from the mean of the three phases of any factor have, owing to association with similar factors, mean genotypic values given by the formula

$$\begin{aligned} I &= i + \frac{A}{1-A} \cdot \frac{iP - kR}{p}, \\ J &= j - \frac{A}{1-A} \cdot \frac{p-q}{2pq}(iP - kR), \\ K &= k - \frac{A}{1-A} \cdot \frac{iP - kR}{q}, \end{aligned}$$

when  $i, j, k$  are the deviations in the absence of association,  $A$  measures the degree of association produced by assortative mating:  $p, q$  are the gene frequencies, and  $P, R$  the corresponding phase frequencies for the homozygous phases.

Writing  $j=i$  to represent complete dominance, and  $P=p^2$ ,  $R=q^2$ , since

$$(p^2 + 2pq)i + q^2k = 0,$$

$$\frac{i}{q^2} = -\frac{k}{p(p+2q)} = \frac{i-k}{1} = \frac{p^2i - q^2k}{2pq^2};$$

and since  $i-k=2a$ , we have

$$I = i + \frac{A}{1-A} \cdot 4aq^2,$$

$$J = i - \frac{A}{1-A} \cdot 2aq(p-q),$$

$$K = k - \frac{A}{1-A} \cdot 4apq;$$

or

$$I - J = 2a \cdot \frac{A}{1-A} \cdot q,$$

$$J - K = 2a \left( 1 + \frac{A}{1-A} q \right).$$

If now the survival factors of the three phases are  $a$ ,  $b$ ,  $c$ , the effect of one generation's selection is given by

$$\frac{p_1}{q_1} = \frac{p_0}{q_0} \frac{ap + bq}{bp + cq} = \frac{p_0}{q_0} (1 + p\overline{a-b} + q\overline{b-c}),$$

since  $a$ ,  $b$ , and  $c$  are near to 1;

hence

$$a = p(a-b) + q(b-c).$$

Now as  $I-J$ ,  $J-K$ , the mean differences in any trait due to a single factor, are small compared with the whole variation within the population, we must take  $a-b$ ,  $b-c$  proportional to  $I-J$  and  $J-K$ . In other words,

$$a-b = (I-J)\gamma,$$

$$b-c = (J-K)\gamma,$$

where  $\gamma$  measures the intensity of selection per unit change in the trait.

Hence

$$a = \gamma(p\overline{I-J} + q\overline{J-K})$$

$$= \gamma \cdot \frac{2a}{1-A} \cdot q.$$

The general case of uniform genotypic selection when the mean values of the phases are modified by homogamy, therefore, reduces to the case already considered in which homogamy is absent. The total effect of homogamy is to increase the effect of selection by the factor  $\frac{1}{1-A}$ . The distribution of frequency ratios is unaltered, for although by introducing a difference between  $I$  and  $J$  the selective effect is made more intense when

$p$  is large, which would tend to make the distribution more symmetrical, this effect is exactly balanced by the increased effect of selection when  $p$  is small. The dominance ratio is therefore unaltered by the direct effect of assortative mating.

#### SUMMARY.

The frequency ratio of the allelomorphs of a Mendelian factor is only stable if selection favours the heterozygote: such factors, though occurring rarely, will accumulate in the stock, while those of opposite tendency will be eliminated.

The survival of a mutant gene although established in a mature and potent individual is to a very large extent a matter of chance; only when a large number of individuals have become affected does selection, dependent on its contribution to the fitness of the organism, become of importance. This is so even for dominant mutants: for recessive mutants selection remains very small so long as the mutant form is an inconsiderable fraction of the interbreeding group.

The distribution of the frequency ratio for different factors may be calculated from the condition that this distribution is stable, as is that of velocities in the Theory of Gases: in the absence of selection the distribution of  $\log \frac{p}{q}$  is given in fig. 1. Fig. 2 represents the case of steady decay in variance by the action of random survival (the Hagedoorn effect).

Fig. 3 shows the distribution in the somewhat artificial case of uniform genetic selection: this would be the distribution to be expected in the absence of dominance. Fig. 4 shows the asymmetrical distribution due to uniform genotypic selection with or without homogamy.

Under genotypic selection the dominance ratio for complete dominance comes to be exactly  $\frac{1}{2}$ , in close agreement with the value obtained from human measurements.

The rate of mutation necessary to maintain the variance of the species may be calculated from these distributions. Very infrequent mutation will serve to counterbalance the effect of random survival; for equilibrium with selective action a much higher level is needed, though still mutation may be individually rare, especially in large populations.

It would seem that the supposition of genotypic selection balanced by occasional mutations fitted the facts deduced from the correlations of relatives in mankind.



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# THE RESEMBLANCE BETWEEN TWINS, A STATISTICAL EXAMINATION OF LAUTERBACH'S MEASUREMENTS

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## INTRODUCTORY

In 1919 the author called attention (FISHER 1919) to the remarkable conclusions which appeared to flow from the measurements of twins obtained by THORNDIKE (1905) from the New York schools. At that time this was the only considerable body of measurements available, and although physical measurements were given for only 39 pairs, of which 8 were of unlike sex, the discrepancy between the results and those of the accepted biological theory was so sharp that its statistical significance was not in doubt. The main points of discrepancy were as follows:

(a) The twins of unlike sex showed an average correlation of 0.78, a value much above the value 0.50 to be expected from children related fraternally; since the average of all twins was 0.85, it appeared that twins of unlike sex were not appreciably less alike than twins of like sex. This fact, if it stood alone, would merit little statistical weight, since only eight pairs of unlike sex were available.

(b) The degree of resemblance between each pair in each particular measurement agreed in its curve of distribution remarkably closely with the curve to be expected theoretically from homogeneous material. If some twins differed from others in their closeness of genetic kinship, it was to be expected that this heterogeneity should make the group of values more variable than it was actually found to be.

(c) Twins chosen as especially alike in one trait were found, when other traits were compared, to show no more than the average degree of resem-

blance. THORNDIKE had not failed to remark this surprising fact, which he described by the term "specialization of resemblance." The correlation between the measure of resemblance of the same pair of twins in different traits, was found in THORNDIKE's material to be  $-0.016$  with a probable error of only  $\pm 0.028$ , suggesting that it was in fact zero, and not distinctly positive as was to be expected.

It appeared at the time that there was no alternative but to accept the facts as THORNDIKE had found them, and to attempt to modify the accepted theory. The valuable and extensive data obtained by Professor LAUTERBACH (see foregoing article) seem to render any such modification unnecessary, and to provide for the first time a quantitative demonstration of the different degrees of resemblance between twins of different physiological origin.

It is not indeed possible from an examination of the measurements to divide a group of twins into two distinct classes as "fraternal" and "identical," and so long as this is the case it were perhaps premature to say that all is plain sailing with the theory of twins. The main difficulties, however, raised by THORNDIKE's data are replaced in LAUTERBACH's much more extensive series of measurements by positive confirmation of the accepted theory of uniovular and biovular twins.

#### CORRELATION BETWEEN TWINS OF UNLIKE SEX

LAUTERBACH's data are sufficiently extensive to determine the correlation between twins of unlike sex with some accuracy. Of the 63 cases, 53 are complete for the four traits, stature, stem length, weight and cephalic index. The great difficulty is that all the pairs are of different ages, and that for the first three traits the growth over the age interval concerned, 7.5 to 19 years, is far from uniform. All three traits show a well marked maximum growth rate, the age of most rapid growth being about 2 years earlier in girls than in boys. The changing growth rate renders the partial correlation eliminating age almost meaningless. A procedure which gets over this difficulty is to fit cubic regression formulae, respectively, to the whole group of boy twins and to the whole group of girls. The deviations from such regression formulae may, as I have shown elsewhere (FISHER 1924), be treated as homogeneous deviates in order to obtain a valid estimate of the correlation. It is still possible that the correlation for twins of mixed sex will be less than that of fraternal twins of the same sex, for factors affecting early or late maturity will inevitably, during the growing period, act less similarly upon twins of unlike sex than on those of like sex; nevertheless, the procedure should be adequate to give a

definite answer to the question whether twins of unlike sex are or are not more alike than ordinary brothers and sisters are known to be.

The sums of squares and sums of products of these deviations are shown in table 1.

TABLE 1  
*Correlation in twins of unlike sex.*

	SUM OF SQUARES		SUM OF PRODUCTS	CORRELATION COEFFICIENT	NUMBER OF CASES
	Boys	Girls			
Stature (mm).....	329,947	358,500	156,441	.4549	63
Stem length (mm)...	50,204	84,206	29,979	.4611	53
Weight (lb).....	14,527	13,813	5,386	.3802	62
Cephalic index.....	.....	....	... ..	.5370	63

The cephalic index shows no age or sex differentiation; the mean value was 806.47 and the variance 1339, the correlation for the boy-girl twins being that given in table 1.

It is apparent that the values agree well with the value to be expected if twins of unlike sex are related in the same manner as ordinary brothers and sisters; the average correlation, 0.4583, with a standard error about  $\pm 0.053$ , agrees sufficiently well with the usual values, about 0.50, even if we make no allowance for the possibility that the correlation in weight is not really so high as are the correlations in the skeletal measurements.

The contrast with THORNDIKE's data is not due to difference in treatment. If we take out individual measures of resemblance as was done for THORNDIKE's data, we obtain the median values for stature 0.585, for stem length 0.405, for weight 0.474, and for cephalic index 0.454, the average being 0.480 and the median of all 241 values being 0.500. These latter estimates are not so accurate as those obtained above, since they depend only on the ratios of the deviations, and ignore their actual values; they simply demonstrate that the two methods yield concordant results. We thus have in these data the first experimental verification of the belief that twins of unlike sex show the same degree of resemblance as do ordinary brothers and sisters.

#### AVERAGE CORRELATION BETWEEN TWINS OF LIKE SEX

For twins of like sex both measurements will be referred to the same standard, representing the mean measurement for that age in the sex concerned; we may, therefore, use HARRIS's (1909) abbreviated method of calculation of intraclass correlations. We take the difference in the measurements for each pair of twins, and find the mean square of these

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differences; dividing this by the mean variance at fixed age, we have  $2(1-r)$ , where  $r$  is a trustworthy estimate of the correlation. In these intraclass correlations the effect of age is only involved in the estimates of the mean variance at fixed age.

TABLE 2  
*The mean square differences between twins of like sex.*

	STATURE IN MILLIMETERS	STEM LENGTH IN MILLIMETERS	WEIGHT IN FOUR-OUNCE UNITS	CEPHALIC INDEX
Boys.....	3316	1492	2261	904
Girls.. . . .	2355	863	1358	542
Together. . .	2826	1180	1800	720

TABLE 3  
*The mean variances at fixed age in twins of like sex.*

	STATURE IN MILLIMETERS	STEM LENGTH IN MILLIMETERS	WEIGHT IN FOUR-OUNCE UNITS	CEPHALIC INDEX
Boys.....	6068	1314	3134	1313
Girls . . . .	4631	1463	3644	1365
Together.....	5339	1388	3393	1339

TABLE 4  
*Correlations in twins of like sex, derived from the data of tables 2 and 3.*

	STATURE	STEM LENGTH	WEIGHT	CEPHALIC INDEX
Boys . . . .	.7268	.4323	.6393	.6558
Girls	.7457	.6891	.8137	.8015
Together... .	.7353	.5749	.7347	.7310

For stature, weight and cephalic index the correlations, 0.73 to 0.74, are very substantially higher than for twins of unlike sex; for stem length the values, especially that for boys, show no such considerable difference. In this connection it should be mentioned that if the like-sex twins are of two kinds, showing, respectively, "fraternal" and "identical" degrees of resemblance, the mean square differences will be dominated by the former group and the random-sampling errors will be very considerably greater than with homogeneous material.

It is thus possible to ascribe the lower values for stem length to a few exceptionally large unlike deviations among fraternal twins of like sex.

On the other hand, it is definitely impossible to regard the like-sex twins as homogeneous in respect of degree of resemblance with the twins of unlike sex; for in this case the correlations for like-sex twins would not differ from those of twins of unlike sex by more than the random-sampling errors to be expected from homogeneous material. These errors have been studied in detail; the standard error of the correlation coefficient as ordinarily quoted does not provide a reliable test, but, as I have explained elsewhere (FISHER 1925), an accurate test is possible by means of the related quantity,  $z$ , which may be regarded as a transformal correlation.

For stature the values of  $z$  are compared in table 5.

TABLE 5  
*Values of  $z$  for statures of like- and unlike-sex twins.*

	$r$	$z$	RANDOM-SAMPLING VARIANCE
Like-sex.....	.1353	.9402	.006969
Unlike-sex.....	.4549	.4909	.016667
Difference. ....		+ .4493	.023636

The difference in  $z$  is 0.4493 with a standard error  $\pm 0.1537$ . Treating the other values similarly we have the results shown in table 6.

TABLE 6  
*Differences of like- and unlike-sex twins with respect to the quantity,  $z$ .*

	STATURE	STEM LENGTH	WEIGHT	CEPHALIC INDEX
Difference in $z$ ...	+ .45	+ .16	+ .54	+ .33
Standard error..	$\pm$ .15	$\pm$ .17	$\pm$ .15	$\pm$ .15

All save stem length show significant differences, and in stem length the difference, though not significant is in the same direction as the others. It is thus obvious that the like-sex twins do not form material homogeneous with those of unlike-sex, but that some or all of them are much more highly correlated. The correlations from LAUTERBACH's data are considerably lower than those from THORNDIKE's data ( $r=0.80$ ), even when we include in the latter one case in five of unlike sex. This suggests that THORNDIKE encountered a considerably higher proportion of identical twins, in addition to a group of twins of unlike sex with unusually close resemblance.

## HETEROGENEITY OF TWINS OF LIKE SEX

In view of THORNDIKE's data which showed no heterogeneity among twins of like sex, it is important to determine if these data show heterogeneity. The most direct test depends on the differences in the measurements of like-sex twins. If  $d$  is the difference of any one pair, found by subtracting the less measurement from the greater, and  $\bar{d}$  stand for the mean difference,  $\bar{d}^2$  for the mean of the squared difference, then for a large sample of normally distributed values we should have

$$\overline{d^2} = \frac{\pi}{2} \bar{d}^2$$

whereas, for a mixture of two such populations, with different mean differences,  $\overline{d^2} - \frac{\pi}{2} \bar{d}^2$  should be positive. To utilize this fact it is necessary to know the standard error of  $\overline{d^2} - \frac{\pi}{2} \bar{d}^2$  and this is found to be

$$\frac{\overline{d^2}}{\sqrt{n}} (2\pi - 6) = \frac{\bar{d}^2}{\sqrt{n}} \times .5321$$

Applying this test to LAUTERBACH's data, I find the values given in table 7.

TABLE 7  
*Tests for heterogeneity in like-sex twins.*

	STATURE IN MILLIMETERS		STEM LENGTH IN MILLIMETERS		WEIGHT IN FOUR-OUNCE UNITS		CEPHALIC INDEX PER MILLE	
	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls
$n$	71	74	66	65	70	73	68	70
$\bar{d}$	40.479	34.622	26.273	17.923	33.286	26.260	22.544	17.410
$\bar{d}^2$	3316	2355	1492	863	2261	1358	904	542
$\overline{d^2} - \frac{\pi}{2} \bar{d}^2$	+742	+472	+408	+359	+521	+274	+106	+66
S.E.	±209	±146	±98	±57	±144	±85	±58	±34

In all cases there appears to be significant evidence of heterogeneity; in the case of cephalic index the separate values for boys and girls are scarcely significant, but taking boys and girls together they provide significant evidence.

Is this apparent heterogeneity due to heterogeneity of origin? At first sight other causes cannot be excluded. The data are admittedly hetero-

geneous in respect of age, and the fact that we are using deviations from fitted regression curves on age will doubtless introduce some further heterogeneity. The effect ascribable to these two causes may perhaps best be estimated by treating the twins of unlike sex in the same manner. For these the results are given in table 8.

TABLE 8  
*Tests for heterogeneity in unlike-sex twins.*

	STATURE IN MILLIMETERS	STEM LENGTH IN MILLIMETERS	WEIGHT IN FOUR-OUNCE UNITS	CEPHALIC INDEX PER MILLE
$n$	63	53	62	63
$\bar{d}_2$	60.048	30.434	48.852	30.937
$d$	5961	1406	4462	1348
$\bar{d}^2 - \frac{\pi^2 d^2}{2}$	297	-49	713	-155
S.E.	$\pm 400$	$\pm 103$	$\pm 301$	$\pm 90$

Two of the deviations are positive and two negative; only that for weight is significant, and this is just the trait in which the effect of age heterogeneity should be most marked. The evidence speaks decisively in favor of the view that twins of like sex are heterogeneous in their mode of origin, while those of unlike sex are apparently homogeneous.

On the theory of fraternal and identical twins about 40 percent of those of like sex should be identical. We cannot assume this ratio *a priori* for the particular sample measured by LAUTERBACH. The correlations indicate, for example, that a larger proportion of the girl twins are identical than of the boys.

The proportion identical in the whole group of boys and girls, must be nearly the same for all measurements, and would be absolutely the same if all cases had been completely measured; if we assume that the standard difference is the same for fraternal twins of like sex as it is for twins of unlike sex we may use the figures for cephalic index to estimate the proportion of identical twins present. If  $a$  is the standard difference for identical twins, and  $p$  the proportion identical, we shall have

$$pa + (1-p)36.715 = 24.997$$

$$pa^2 + (1-p)1348 = 720.38$$

whence,

$$a = 16.856$$

$$p = .59007$$

If we adopt the value 59 percent identical, we may infer the correlation in the identical group from the actual correlations of the like-sex pairs,

taking the average value for the unlike-sex pairs, 0.4583, to represent the fraternal correlation; in this way we obtain

	<i>Stature</i>	<i>Stem length</i>	<i>Weight</i>	<i>Cephalic index</i>
Correlation for identical twins	.9278	.6898	.9268	.9205

Apart from stem length the agreement is excellent.

The values obtained for the estimated correlation between identical twins are of great interest, since, if they could be determined with certainty, they would afford a direct measure of the importance of genetic factors in determining these traits. From an examination of PEARSON'S data for the correlation of related adults the author concluded (FISHER 1918) that, if heredity is due to a number of cumulative Mendelian factors, more than 90 percent of the variance must be due to genetic causes. PEARSON'S data referred to stature, span and cubit, and LAUTERBACH'S data not only confirm the fact for stature, but make it probable that it should be extended to cephalic index, and, for children at least, to weight. It should be remembered that the differences observed in these identical twins are absolutely small. The standard difference in stature is only 26 mm, and from my own experience of measuring children a standard error of measurement of 3 to 5 mm would not seem excessive. The effect of random errors of measurement will be to lower the correlations and will be especially important for stem length, when the measurement errors are at least as great as for stature, while the absolute differences are much less. For cephalic index the standard difference is only 17 parts per mille, and an error of 1 mm in head breadth will produce an error of nearly 6 parts per mille. The average of the four correlations as estimated for identical twins, 0.894, will therefore have been subjected to a dilution of uncertain amount due to errors of measurement.

#### SPECIALIZATION OF RESEMBLANCE

THORNDIKE'S data clearly indicated that twins more alike in one character were not, on the whole, more alike in other characters. This conclusion is so contrary to the accepted hypothesis that, if it were substantiated, that hypothesis must be abandoned.

In the present data three of the characters, height, stem length and weight, are somewhat closely associated together, and the best test of specialization will be to compare resemblance in stature and cephalic index.

The like-sex pairs were divided according as the difference in stature was 0 to 52 mm, or 53 mm upwards, and also according as the difference in cephalic index was 0 to 25, or 26 upwards. The results are given in table 9.

TABLE 9

Boys				Girls			
Difference in stature				Difference in stature			
	Low	High	Total		Low	High	Total
Difference in cephalic index Low.....	33	10	43	Difference in cephalic index Low.....	40	11	51
High.....	20	5	25	High.....	13	6	19
Total....	53	15	68	Total....	53	17	70

Neither table, nor the two thrown together, gives any indication that those more alike in stature are more alike in cephalic index, or *vice versa*. To test the matter more exactly, the mean square difference in stature and cephalic index was found for each class (table 10).

TABLE 10

*Mean square difference in stature.*

Boys				Girls			
Difference in stature				Difference in stature			
	Low	High	Total		Low	High	Total
Difference in cephalic index Low.....	598	13165	3521	Difference in cephalic index Low.....	444	9634	2426
High.....	869	11911	3077	High.....	899	6218	2578
Total....	700	12747	3358	Total....	555	8428	2467

With boys, those differing greatly in cephalic index have on the average a somewhat less difference in stature, than those with similar cephalic indices; with girls, the reverse is true, but to a less extent. If, however, we confine attention to those with low differences in stature, so excluding the high values which tend to dominate the averages, both sexes show a distinctly lower difference in stature, for the children with similar cephalic indices. Putting the two sexes together we have

	Cases	Mean square
Like in cephalic index.....	73	516
Unlike in cephalic index.....	33	881

This difference is just over the verge of significance; by my  $z$  test (FISHER 1925, chapter VII) I find  $z = 0.267$ , while the value 0.234 is exceeded by chance in only 5 percent of cases.

Repeating the test for differences in cephalic index, we have the grouping shown in table 11. Again, in the totals, the boys alike in stature show actually greater divergence in the cephalic index, while the girls show the

TABLE 11  
Mean square difference in cephalic index

Boys				Girls					
Difference in stature				Difference in stature					
Difference in cephalic index		Low	High	Total	Difference in cephalic index		Low	High	Total
	Low . . . .	156	203	167		Low . . . .	122	227	145
	High . . .	2382	1296	2173		High . . .	1494	1853	1607
	Total . .	1000	568	904		Total . . .	458	801	542

reverse to a less extent. Confining the comparison to those alike in cephalic index, the two sexes agree, and give

	Cases	Mean square
Like in stature . . . . .	73	137
Unlike in stature . . . . .	21	216

By the  $z$  test,  $z=0.228$ , and the 5 percent point is at 0.266.

Both results, therefore, are on the verge of significance, and together seem to establish that, if we exclude the cases of wide discrepancy, twins much alike in head form tend to be more alike in stature, and *vice versa*.

#### CONCLUSIONS

LAUTERBACH's physical measurements of about 200 pairs of twins appear to provide unequivocal evidence for the following conclusions:

(1) Twins of unlike sex resemble each other to approximately the same extent as do ordinary brothers and sisters.

(2) Twins of like sex show on the average a considerably closer resemblance.

(3) Twins of like sex are heterogeneous, and are therefore divisible in respect of resemblance into at least two classes.

(4) The data may be interpreted as due to a mixture of identical and fraternal twins, of which about 59 percent appear to be identical. The correlations between identical twins must, on this supposition, be about 0.9 or over.

(5) If we set aside twins with large differences in stature as certainly fraternal, the remainder show that those with large differences in cephalic index have on the average larger differences in stature; *mutatis mutandis*, the same is true of cephalic index. The data thus supply, for the first time, evidence of association of resemblance in different traits.



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## ON SOME OBJECTIONS TO MIMICRY THEORY; STATISTICAL AND GENETIC

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### I. INTRODUCTORY.

THE great statistical interest of all applications of selection theory, of which mimicry is certainly one of the most extensive and detailed, makes the validity of the latter a matter of importance even beyond the limits of the biological sciences. Moreover, it appears to be probable that disputed points which have arisen in discussions of mimicry, should, in so far as they are of a purely statistical nature, be capable now of a definite and final decision. The importance of inheritance in selection theory has led to a study of the statistical effects of Mendelism, with the result that the genetical arguments are in nearly the same position as the purely statistical. It is now becoming increasingly widely understood that the bearing of genetical discoveries, and in particular of the Mendelian scheme of inheritance, upon evolutionary theory is quite other than that which the pioneers of Mendelism originally took it to be. These were already, at the time of the rediscovery of Mendel's work, in the full current of that movement of evolutionary thought, which in the nineties of the last century, had set in in favour of discontinuous origin for specific forms. It was natural enough, therefore, that the discontinuous elements in Mendelism should, without sufficiently critical scrutiny, have been interpreted as affording decisive evidence in favour of this view. No attempt will here be made to give an extended account of the general bearing of Mendelism upon evolutionary theory; it should, however, be borne in mind that the reinterpretation of the significance of Mendelism in cases of mimicry is but part of a more general recovery of genetical opinion from positions adopted at a somewhat immature stage of the development of that science.

It seems to the author imperative, in undertaking such a task, the value of which depends solely upon its logical cogency, to insist upon the fact that, in criticising biological conclusions, the criticism is solely aimed at their supposed relation to the observations upon which they are based, and is in no way opposed to, or confirmatory of, these observations. Mathematical symbolism will be avoided; nevertheless the work will be mathematical in the sense that for the sake of certainty and precision upon the points discussed, innumerable side issues, some possibly of importance, will be totally disregarded, and the biological facts utilised will be reduced to a mere abstract of their real complexity.

### II. SUPPOSED STATISTICAL LIMITATIONS OF MÜLLERIAN MIMICRY.

Certain *a priori* statistical limitations are supposed to inhere in the theory of Müllerian mimicry; these have been most lucidly developed by G. A. K. Marshall (1). Marshall suggests that, for arithmetical reasons, of two equally unpalatable species inhabiting the same region, the less numerous will tend to resemble the more numerous, while the more numerous will not reciprocate

this tendency. (Marshall does not suggest that the more numerous will tend to decrease the resemblance.) The general purport of his paper is to emphasise the Batesian as opposed to the Müllerian factor in mimicry, and that principally for biological reasons, with which only a professed entomologist should deal. The question, however, as to whether there is or is not such a statistical limitation as Marshall claims to the scope of the Müllerian factor, is of the type to which I suggest mathematical reasoning may profitably be applied.

When illustrated by hypothetical type cases nothing is clearer than the distinction between the Batesian and the Müllerian factors; if, however, we ask exactly where the line is to be drawn, it is not too easy to give an answer in terms directly referable to the actual bionomic situation. It is admitted that between two equally unpalatable species only the Müllerian relation is possible, and between a species, which would never be unwelcome (if such exist), and one which would never be attacked, any mimicry must be Batesian; but these are extreme possibilities and Marshall insists, as I hope to show, rightly, that the existence of intermediate degrees of palatability gives increased scope to the Batesian factor. I shall also suggest that in cases involving such intermediate degrees, the Müllerian factor cannot properly be excluded, and that the distinction between the two agencies cannot be drawn offhand without further consideration.

If we imagine three species, occupying the same region, of which A is highly unpalatable, B less so, while C is free from objectionable qualities, then all possible situations may be exhaustively classified as follows (I, II, III, IV), in which, however, the alternative reasons given for the occurrence of any situation ( $a, b, c$ ), are by no means exhaustive, but might conceivably be much elaborated by detailed biological observations.

I. A, B and C all liable to attack.

II. B and C liable to attack, but not A.

(a) Wise and experienced birds, knowing full well the flavours of each species, are hungry enough to attack B, but not hungry enough to attack A.

(b) Birds have attacked A and found it to be unpalatable, but have not yet had sufficiently impressed upon their minds the unpalatable qualities of B.

III. A and C liable to attack, but not B.

(c) Birds have attacked B and found it to be unpalatable, but have not yet had sufficiently impressed upon their minds the unpalatable qualities of A.

IV. C only liable to attack.

In situation I, mimetic resemblance is without effect, while in situation IV, C would gain by being mistaken for A or B, and A or B would lose by being mistaken for C. C might, therefore, if the former effect were to exceed the latter, become a Batesian mimic of A or B, while, on the contrary, A and B would gain by emphasising their distinctive colouring, if so they could diminish the danger of confusion with C. Similarly, in situation II, A loses by being mistaken for B, and B gains by being mistaken for A; while in situation III the reverse is the case; both A and B will seem, therefore, to be acted upon by opposing tendencies, one tending towards similarity, and the other towards dissimilarity. It is only when the possible situations are analysed into their suggested causes that it is possible to indicate the resultant effect.

For this purpose we distinguish the "Batesian situation" II (a), from the "Müllerian situations" II (b) and III (c), recognising that this classification need not be exhaustive. It is then seen that Batesian situation is to be distinguished by (i) depending upon differences of unpalatability and (ii) producing a "Batesian tendency" for B to approach A and for A to recede from B. While

the Müllerian situations (b) and (c) do not depend on any difference of unpalatability, but are taken to occur whenever both species are, on occasion, deemed inferior to some alternative food. It is not, however, obvious from the above analysis that the net effect of (b) and (c) will be to cause a mutual though possibly unequal approach between the two species; such a mutual tendency to approach will be called a "Müllerian tendency." It is Marshall's contention that when the unpalatability is equal, the less numerous species will be attracted by the greater, but the greater will not be attracted by the less. Marshall does not fail to draw from this conclusion a very important consequence, for, as he points out, his premises lead to the inevitable conclusion that, when a mimetic similarity is once effected, the larger species will have gained the smaller share, but still a share, of advantage from the association, and one might be inclined to argue from this that the larger species also will be led to approach this more advantageous condition. The far-reaching conclusion is drawn that such an argument is not valid, unless a continuous path from the first state to the second can be shown to exist, such that the advantage increases for each step along the path. Such a conclusion, if correct, would throw upon the selectionist an onus of detailed demonstration, which his opponent might increase indefinitely by challenging the details with increasing minuteness. Even if the case of Müllerian mimicry were not in itself of sufficient importance, it would be essential to examine in some detail the particular case in which the argument from ultimate advantage is believed to lead to an erroneous conclusion.

Marshall's argument is essentially as follows: if A and B are two equally distasteful species, of which B is the less numerous, then, in the absence of mistakes due to the resemblance of the two species, the young birds will take a proportionately heavier toll of B than of A, before they have all learnt their lesson; consequently any mutant of A which resembles B will suffer more than the non-mutant type, and in consequence will be eliminated. It will be seen that the mutant is supposed to lose the whole of the advantage of the warning colour A, and in return to receive only the less advantage of the warning colour B, and this argument is indeed conclusive in showing that a mutation, which leaps clear outside the protective influence of its type, will suffer heavily for its rashness, even if, miraculously enough, its leap lands it in the heart of the protective influence of a less numerous aposeme. But what of a less violent mutation? Is it possible to gain some of the advantage of resembling B without losing the whole of the advantage of resembling A? Is it even possible that increased shelter from aposeme B will more than counterbalance the loss from decreased shelter from aposeme A? In his answer to Marshall's argument Dixey (2) puts forward a directly opposite supposition, namely that a mutant of appearance intermediate between A and B, would gain the full advantage of both resemblances. In fact, whereas Marshall assumes that the whole of the advantage of resembling A is lost before any of the advantage of resembling B is gained, Dixey assumes, on the contrary, that the whole of the advantage of resembling B may be gained before any of the advantage of resembling A is lost. Both are clearly extreme assumptions; neither can be true generally, and since the two assumptions lead to opposite conclusions it would seem, as far as these arguments carry us, that we are faced with a balance of forces of unknown magnitude, and can neither assert that the Müllerian principle will work, nor that it will fail.

There remains the argument upon which Müller relied, that the final condition of close resemblance being beneficial to both species, both will therefore tend to approach this advantageous condition. Marshall challenges the legiti-

macy of this argument, his reason being the decisive one that he has disproved the conclusion in a particular instance; as we have seen, however, Marshall's argument in the chosen instance is indecisive, and the general argument from the advantage of the final state is in a position to reassert its claims. If it is true, however, it should be possible to devise a form of argument which shall show unequivocally, on the agreed postulates, that the admitted Müllerian situation will in fact produce a Müllerian evolutionary tendency affecting both species concerned.

Such an argument may, I suggest, be constructed by comparing the fate of any deviation from the type A, not with the average type, but with an equally conspicuous but opposite deviation. It will be admitted that variations of the species A, whether due to mutation or to Mendelian recombination, will be equally frequent in the direction of B as in the opposite direction; we may, therefore, without error, consider such variations to occur in pairs comprising variations of equal magnitude, but in opposite directions. Since they are of equal magnitude they will lose (if anything) equally by failing to be recognised as typically A, but if either, or both, are ever mistaken for species B, the greater benefit will certainly be reaped by the variation in the direction of B. Since the whole species may be regarded as made up of such pairs of variations, and since in every pair selection favours the one more like B, if either is favoured, the net resultant must be a modification in the direction of species B.

It will be seen that the condition for the existence of a mimetic tendency is that in a certain proportion of the situations in which A is liable to, but B is immune against attack, members of species A should, through their similarity to B, actually escape attack. This is somewhat different from the condition arrived at by Prof. H. H. Turner, who speaks (7) of an actual overlap of the variations of the two species as the condition for the efficacy of Müller's statistical argument. The possibility of error on the part of the predator seems an essential feature in mimicry theory, and allowance can be made for it in Turner's treatment, provided we interpret his distribution curves as referring, not to the objective variability of the species, but to the (probably much greater) variability of the predator's subjective impressions, influenced as these must often be by inattention or haste, and by deceptive or insufficient illumination—in fact, by whatever circumstances conduce to error, human or avian. It is rather remarkable that, on a subject so remote from direct evidence as the subjective impressions of birds, we should possess three good reasons for assuming an approximately normal distribution: (a) that the reasons for which this distribution is chosen as the "normal law of errors" can scarcely be confined to mankind, (b) that the objective variability of a measurable character due either to Mendelian segregation, or to environmental fluctuations, is usually closely normal, and (c) that the resultant compounded of two independent distributions is necessarily more normal than one, and possibly than both of its components.

The argument above developed may assuredly be refuted by disproving any of the biological factors assumed in the discussion. If it were proved that situations never in fact arise in which a member of A would survive if mistaken for B, but would perish if not so mistaken, that no predator learns by experience or is ever influenced by mimetic resemblances, or that such variations of A as do favour the resemblance are not heritable, then the Müllerian theory of mimicry would fail as an explanation of the resemblances observed. The sole point established by the above reasoning is that if these biological factors are admitted the resulting evolutionary tendency cannot be confined to the less numerous of two species. The efficiency of Müllerian selection will doubtless be greater

(*ceteris paribus*) with the smaller species, but the supposed statistical objection to the Müllerian attraction of a larger species (or group) by a smaller is wholly fictitious.

### III. THE THEORY OF SALTATIONS.

Punnett (3, Chap. VI, pp. 72-74) repeats Marshall's argument, and concludes without reservation that Müllerian mimicry of a less numerous by a more numerous species is excluded by it. At first sight the argument appears irrelevant to Punnett's main contention of the inadequacy of Natural Selection to produce adaptations, for he evidently, unlike Marshall, would reject also both Batesian mimicry, and the Müllerian mimicry, of the more numerous by the less numerous species. Nevertheless, it would not be altogether fair to regard Punnett's citation of Marshall's argument as a merely extraneous addition to his indictment, such as by arousing suspicion of error, though on an irrelevant issue, might serve to secure a verdict on the main count; on the contrary Marshall's argument plays a small yet essential part in his destructive argument derived from mimicry rings. The case of two presumably palatable female types each quite unlike the corresponding males, which males are unlike each other, is chosen to illustrate this difficulty. The two females show an apparent mimetic resemblance to three other butterflies, two regarded as definitely unpalatable and the third as doubtfully so. Assuming that the non-mimetic males represent the former appearance of the two mimetic females, it is asked how the latter have come to resemble the distasteful members of the ring. Granted that these models might once have been not unlike in appearance to one of these males it can scarcely be assumed that they ever resembled both, either simultaneously or consecutively; but unless such a resemblance formerly existed a *gradual* mimetic evolution is precluded, and we should be forced to admit that the mimetic females arose as sports or saltations totally unlike their mothers. (The word "saltation" is used here in preference to "mutation" since the modern Mendelian usage of the latter word contains no suggestion of a pronounced difference. Mutations may be changes quite small compared to the individual variability of the species.)

It will be seen that for Punnett's argument on this important point, the gradual and mutual convergence of two or more different warning colours must be wholly excluded, for if the possibility of such a process is admitted the difficulty of imagining a continuous sequence of changes entirely disappears, while on the contrary the assumption of discontinuity becomes a burden upon the theory, involving as it does the definite improbability of hitting off a good resemblance at one shot. Consequently Marshall's argument, which Punnett seems to have taken as reimposing all the limitations of the Batesian situation, plays an essential part in the argument in favour of saltations; so essential indeed that it seems impossible to repair the breach made by its removal.

The case for saltations as presented by Punnett was not entirely negative and destructive in character; it embodied one (then) recently discovered fact of considerable interest, namely that the differences between the three forms of the trimorphic female of *Papilio polytes* could be ascribed to two Mendelian factors, both limited in their obvious effects to the female sex, and one apparently necessary for the manifestation of the second.

This fact is of importance as indicating the mechanism by which a clear polymorphism is maintained; it shows that polymorphism in this case, and probably in similar cases, is dependent on one or more Mendelian factors the function of which is to switch on one or other of the possible alternatives, just

as the more widespread dimorphism of sex is also dependent upon the Mendelian mechanism. In some groups, e.g. *Drosophila* and Man, a whole chromosome is utilised in the process of sex determination, in some fishes, on the contrary, (6), crossing over has been found to occur between the "sex-chromosomes" in the male (the heterogametic sex in this group), and we evidently ought more properly to speak of the sex gene rather than the sex chromosome as the agent of sex determination. The passage from the one condition to the other, by the cessation of crossing over, presents no inherent difficulties, especially as Mendelian factors are known which expedite or inhibit crossing over. The reason for such a change is not so obvious, but since both systems are found still in use, it is probable that each has, upon particular conditions, its own advantages.

The core of Punnett's argument in favour of the production of mimetic forms by saltations lies in the Mendelian behaviour of the polymorphic females, for it is argued that these Mendelian factors must have arisen originally as mutations, and seeing that the different forms demonstrably differ by only single factor differences, these types must have sprung into existence each at a single leap. Convincing as this argument at first sight seems, we should, nevertheless, at once recognise our folly if we argued that because the sex difference in *Lebistes* is apparently determined by a single factor, therefore a female fish of that genus, with the appropriate adaptations of her sex had arisen by a single saltation from a male of the same species! Or *vice versa*. In this case we are freed even from the necessity of rejecting the supposed saltation as improbable, for since the reproduction of the species requires the co-operation of both sexes, we may be certain that the origin of the sex factor antedated the evolution of separate sexes, and has persisted, in its function of switch, unchanged during the whole course of the evolutionary development of these two types.

The example of sex emphasises strongly the fact that it is the function of a Mendelian factor to decide between two (or more) alternatives, but that these alternatives may each be modified in the course of evolutionary development, so that the morphological contrast determined by the factor at a late stage may be quite unlike that which it determined at its first appearance. The inference, therefore, that because a single factor determines the difference between a mimetic and a male-like form in *P. polytes*, therefore the mimetic form arose fully developed by a single mutation, is one that cannot fairly be drawn; it requires, in fact, the gratuitous assumption that no evolutionary change has taken place in the two alternative forms since the dimorphism was first established.

Certain genetical experiments have demonstrated that genetic changes of the kind here considered are compatible with a purely Mendelian scheme of inheritance. In rats, the hooded (black and white) pattern is a simple recessive to the "self" or "solid" coloration; the case is probably parallel to the "Dutch" pattern in rabbits, and the "recessive pied" in mice. In studying variations in the hooded pattern Castle (4) found that by selection it was easy to obtain strains of hooded rats which were almost entirely black, and other strains almost entirely white, and equally, of course, a large number of stable patterns of an intermediate character. All these types of "hooded" behaved as before, as simply recessive to self-colour. Two possible explanations were put forward; the first possibility was that the modification produced by selection lay in the hooded gene, that, in fact, selection had sorted out from a large number of slightly differing allelomorphs, those favouring much or little pigmentation, and consequently that the surviving hooded genes had been modified by selec-



tion; the second possibility was that the hooded gene was invariable in character, but that the pigmented area depended also on the co-operation of other genes, so-called modifying factors, and that the change in the hooded pattern was the result of selection, among the alternatives presented by these modifiers, of those types which developed larger or smaller pigmented areas respectively. A crucial experiment was devised to decide between these possibilities. Rats of both selected lines were bred back to unselected selfs, the young were inbred, and the hooded pattern was recovered in the grandchildren; if the modification had taken place in the hooded gene the recovered hooded rats would have received fully modified hooded genes, and must have been as dark as the hooded line from which they were obtained; but, if other factors were responsible, the hooded grandchildren would have received these equally from their self and from their hooded grandparents, and would consequently be less dark than the latter. The second alternative was proved to be correct, the modification being readily transmitted by self-rats which contained no hooded gene. The gene, then, may be taken to be uninfluenced by selection, but its external effect may be influenced, apparently to any extent, by means of the selection of modifying factors.

Unless the above analogies are wholly misleading, we should suppose that the factors H and R which Fryer (5) found to determine the differences between the polymorphic forms of *P. polytes*, each arose suddenly by a mutation, and that the new genes so produced have been entirely unmodified since their first appearances. On the other hand, we should see no reason whatever on genetic grounds to believe that the combination HHrr on its first appearance at all closely resembled the modern form *polytes*, or was an effective mimic of *P. aristolochiae*; nor that the combination HHRR resembled the modern form *romulus*, or was an effective mimic of *P. hector*. The gradual evolution of such mimetic resemblances is just what we should expect if the modifying factors, which always seem to be available in abundance, were subjected to the selection of birds or other predators.

#### IV. STABILITY OF THE GENE-RATIO.

It should be emphasised that there is nothing in the above argument which helps to explain polymorphism itself. The phenomenon is sufficiently uncommon to suggest that it must always owe its origin to some rather special circumstances; however, the Mendelian character of the phenomenon does suggest one short step in the direction of a solution, namely, that the underlying condition for its development is that the proportionate numbers of the genes of some Mendelian factor, having a fairly marked effect, should be in stable equilibrium. By stable equilibrium I wish to imply that, if this ratio is disturbed from its equilibrium value it will automatically tend to return to that value, in whichever direction the disturbance takes place. Both Fryer and Punnett speak of the proportion of the Mendelian types being in stable equilibrium, in the absence of selection, and for particular values of the frequency ratio of the genotypes. Thus it seems to have been believed that the ratio is particularly stable if 44 per cent. are recessive, and this is compared with the observed percentage, 45, as an indication that the species is in a stable state. It should be stated emphatically, firstly, that in the absence of selection the equilibrium of the gene-ratio is neutral, and is not properly described as stable, for on disturbance there will be no tendency to recover its former value; and, secondly, that all possible ratios either for the genes, or for the genotypes are

on exactly the same level, no ratios being specially favoured. The only point to which, in this system, the term stability can properly be applied, is the ratio of the number heterozygous to the geometric mean of the numbers homozygous, and of this ratio there is no experimental evidence available; barring restrictions on random mating of a most drastic character, this ratio can scarcely differ much from its stable value, two.

That a genuine stability probably characterises the gene-ratio in polymorphic species appears probable from the consideration of the time required for a single mutation to increase sufficiently in numbers till it affects, say, one-third of the genes of the species. In the absence of selective advantage this time will be that of a number of generations of the same order as the number of individuals which there are in the species; for many species this would be at least a thousand times as long as the longest time allowable. In order to affect a considerable portion of the population within a reasonable time the new gene must enjoy a selective advantage. If this advantage were to continue, however large a proportion of the population were occupied by the favoured gene, the process would be continued until the extermination of its allelomorph, and no dimorphism would remain. Only if the selective advantage wanes with increasing numbers, disappears at a fixed ratio, and beyond that is reversed, will a true stability of the gene-ratio be established. Stability is thus not only evidence of selection, but of the dependence of selective advantage upon the actual ratio of the alternative types.

Stabilising selection can scarcely be other than exceptional, yet it may be expected to arise in several ways. A Batesian mimic, for example, will receive less protection, the more numerous it is in comparison with its model; a dimorphic Batesian mimic will therefore adjust the numbers of its two forms, if these are dependent upon a single Mendelian factor, until they receive equal protection; any increase in the numbers of one form at the expense of the other would diminish the advantage of the former and increase that of the latter, thus producing a selective action tending to restore the original proportion. Note that a mimic owing its advantage to Müllerian situations only should not be dimorphic unless additional causes of stability are at work, for apart from these the selection produces an unstable equilibrium, from which the ratio will continue to depart until one or other type is exterminated.

A second form of stabilising action is found in reproductive selection. The stable ratio of the sexes is clearly due to this cause, as is that of the thrum-eyed and pin-eyed primroses. It is interesting to note that Fryer, in his breeding experiments with *Papilio polytes* observed numerous cases of sterile unions, which suggested to him the possible existence of "illegitimate" pairings. One of the simplest possibilities of this type is a merely greater fertility of the heterozygous as compared to the homozygous condition. As I have shown elsewhere, (8), the stability in the gene-ratio of factors for which the heterozygote is favoured, affords also a possible explanation of the phenomenon of "hybrid vigour."

It should perhaps be noted that Gerould's work (9), on the dominant white observed in the female of several species of *Colias*, also reveals some peculiar features suggestive of a stability mechanism governing the yellow-white gene-ratio. Gerould reports that great difficulties were encountered in obtaining the homozygous-white types, these difficulties being evidently connected with the occurrence of a closely linked lethal factor. When pure white broods had been obtained, from a strain freed from the lethal, the failure of the males to mate caused the introduction of wild males, and these were found to bring in the

lethal factor. The fact that this particular lethal is not apparently rare in nature, although we should expect it to die out somewhat rapidly, suggests that a stabilising system must be present. The genetic complexities are not fully elucidated, for certain types of mating seem regularly to give an abnormal sex-ratio (3 ♀ : 2 ♂). It is interesting in connection with the modifying effects of selection, that Gerould notes the occurrence of a fluctuating tinge of yellow on the wing of the genetically white female, and ascribes its variability to secondary factors.

Cases such as those of *P. polytes* and *Colias* have an additional special interest, in that the stability mechanism probably involves both the reproductive elements of genetic lethality and sterility on the one hand, and on the other hand, elements involved in the differential adaptation, of the forms concerned, to survival in their wild environments; for if these latter were absent we should expect the gene-ratio to be the same in all localities. The unique possibility therefore presents itself of obtaining a direct measure of the selective value of such differential adaptation by equating it to the calculable effects of the reproductive selections, against which we find it in nature balanced. The importance of such a direct determination need scarcely be emphasised; the hindrances to free reproduction in these groups have appeared hitherto merely as an obstacle impeding the Mendelian analysis of the polymorphic forms; it is much to be hoped that, in view of the application outlined above, their elucidation may, in future studies, be made a principal object of research.

Whatever be the cause to which a factor owes its stability, any species in which a stable factor occurs will be potentially dimorphic, and permanently so unless in changed conditions the stability can be upset. If, in this condition, selection favours different modifications of the two genotypes, it may become adaptively dimorphic by the cumulative selection of modifying factors, without alteration of the single-factor mechanism by which the dimorphism is maintained.

#### SUMMARY.

1. The contention of Marshall that statistical reasons preclude the action of selection from favouring the modification of a more numerous species in the direction of a closer resemblance to a similar but less numerous species, is without valid foundation.

2. The contention of Punnett that mimicry rings containing more than one palatable mimic, much modified from its primitive appearance, must have arisen by discontinuous saltation depends wholly on the validity of Marshall's argument.

3. The Mendelian behaviour of the different forms of a polymorphic species does not prove that these forms arose by single saltations.

4. The stability of the gene-ratios of factors controlling polymorphism implies a selective action, reproductive or other, influenced by the frequency-ratio of different forms. Any factor causing visible differences, and possessing a ratio of stable equilibrium, will provide a potential dimorphism capable of evolutionary development by the selection of modifying factors.

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TRIPLET CHILDREN IN GREAT BRITAIN AND IRELAND.

BY

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*Triplet Children in Great Britain and Ireland.*

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1. *Origin of Material.*

The study of twins is somewhat impeded in Great Britain by the absence of the registration of multiple births; for, contrary to the practice of most civilised countries, the occurrence of twins appears in the registers as two distinct births, occurring to the same parents at an unusually short interval of time. Consequently official data are lacking as to the frequency and sex distribution of twins and triplets. On the other hand, the existence of a Royal Bounty for triplets supplies a potential source of information, of which it is believed scientific use has not previously been made. The records of the Royal Bounty, in the charge of the Secretary of His Majesty's Privy Purse, constitute in effect a special registry of triplet births, including the great majority of the cases in Great Britain and Ireland. With the support of the Medical Research Council the author was kindly allowed access to this material, for which he desires to express his indebtedness to all concerned.

Visits to individual families for the purpose of measuring the surviving children made the enquiry a more expensive one than if the information had been gathered merely by correspondence. During three years the British Association made grants to a Committee, appointed by Section H, under the chairmanship of Dr. Shrubbsall, in aid of this work, which together with a generous donation from Major Leonard Darwin covered nearly the whole of the actual expenditure. This would have been much greater had not a number of gentlemen (see below, p. 288), situated in districts distant from London, freely given their assistance in taking measurements.

A study of the conclusions arrived at in different investigations of twin births

reveals two serious disabilities from which existing bodies of data ordinarily suffer. The most important collections of twin measurements, such as those of Thorndike (10), Dahlberg (3), and Lauterbach (4), refer to twins of ages from childhood to adolescence, with a few adult cases. Statistical allowance for age is not easy over a period which includes a well-marked maximum of growth rate, which occurs at considerably different ages for boys and for girls, and doubtless at different ages also for children of the same sex. Attempts have been made to eliminate the effects of varying age by comparison with a larger number of children from single births (10), data which are seldom available, and by the use of empirical growth curves based upon the twin measurements (4), a procedure which is useful only when the data are exceptionally extensive. Both methods, however, are open to the objection that possibly hereditary factors play an important part in the determination of precocity or the reverse, and that, if so, similar genetic variation in children of opposite sex may produce a materially less degree of resemblance between twins (who, of course, are usually at the same age at the time of measurement) than in the case of twins of like sex.

The second disability also affects collections of measurements, but in an even more serious degree appears to influence studies of the inheritance of the twinning tendency. It is that cases are brought to the notice of the investigator on account of their interesting character. The investigation may even have been initiated by some such interesting group of cases, and even if subsequent cases are collected by some sound method which excludes the possibility of unconscious selection, the collection will still in some degree retain its atypical character. Large collections, if not composite, escape this difficulty better than small collections, but with the increased precision with which tests of statistical significance are now carried out, it becomes the more important to emphasise that only data obtained on a carefully planned and uniform basis are capable under such tests of yielding valid conclusions. How far this consideration is capable of explaining the great discrepancies, not only between the conclusions of different investigators, but between the bodies of data they present, can only be known as material free from this suspicion is accumulated.

The material for the present study comprises three consecutive years of the King's Bounty records, with dates of birth ranging from October 8, 1917, to September 28, 1920. The period was chosen so that from the time the investigation was commenced surviving children should be available for measurement at a fixed age, which was selected in advance at  $6\frac{1}{2}$  years. If two or more triplet children survived to this age, they were measured. Since the limiting factor of the enquiry was the cost of visiting the children at this time, no attempt was

made to recover at a later date the measurements of cases, which for one reason or another could not be measured at the right time. It would have been scarcely possible to obtain the measurements but for the assistance of Mr. E. M. Somerfield in Ireland, Dr. Alexander Low in Scotland, and Mr. L. H. C. Tippet who measured a large number of cases in the Midlands and North of England. Mr. A. R. Stoney Smith also measured several of the earlier English cases. To the care of these voluntary assistants in procuring accurate and comparable measurements must be ascribed the clear and unambiguous results to which the measurements data point.

All parents, whether the triplet children survived or not, were asked to fill in a relationship form, giving the numbers and sexes of all children, whether by single or multiple births, in certain nearly related families. The importance of obtaining complete data over a uniform range of kinship cannot be over-emphasised. In many families remarkable cases of multiple births from comparatively remote relatives are thrust under the notice of the investigator, but must be ignored, since it is only for the families of near relatives of the father and mother of the triplets that complete and reliable information can ordinarily be obtained. The children born to the father and mother of the triplets and the children born to their parents are normally well known; in addition, the questionnaire covered the children of the brothers and sisters of the parents of the triplets. The latter contain a proportion of incomplete families, and a number of individual families had to be omitted owing to migration. Only cases in which the number and sexes of the children could be listed with confidence were included.

The numerical data, together with the specification of the measurements taken, have been filed at the Natural History Museum at South Kensington, under the arrangements made for the conservation of biological measurements by the Measurements Committee of the British Association.

## 2. *Survival.*

In all 166 cases of triplet births were available for enquiry; of these, however, no reply could be obtained in 20 cases, either from change of address since the birth of the children, or more rarely from the parents being unwilling to supply information. The data for survival in the remaining 146 cases are given below.



Table I.—Survival of Triplet Children Born Alive.

Born.	3 boys.	2 boys, 1 girl.	1 boy, 2 girls.	3 girls.	Total.
All living	2	4	8	6	20
One dead—					
Boy	7	4	10	—	21
Girl	—	3	12	6	21
One living—					
Boy	6	8	3	—	17
Girl	—	1	11	9	21
Sex unknown	—	2	2	—	4
All dead	7	15	14	6	42
Unknown	8	3	5	4	20
Total	30	40	65	31	166

The proportion surviving differs in the two sexes, but does not seem to differ greatly in the four types of triplet birth. From the table above the following percentages of survival are calculated :—

Table II.—Percentages Surviving.

	3 boys.	2 boys, 1 girl.	1 boy, 2 girls.	3 girls.	Total.
Boys	39	37	40	—	39
Girls	—	26	51	48	46

The low proportion of survival among the girls, in sets with two boys and one girl, is noticeable, but this is based on only 35 cases, of which 9 survived.

As is to be expected both from the genetical similarity of the children and from the similarity of their experience up to and including birth, the deaths of one or more children in the same set are far from independent. Of 101 pairs of two boys, in 20 cases both survived, in 38 one survived, and in 43 cases neither survived ; thus the chance of survival of a boy is raised to 51 per cent. if his brother lives from the value 31 per cent. if his brother dies. Of 188 pairs of opposite sex, in 40 cases both lived, in 32 cases the boy only survived, in 37 cases the girl only survived, and in 79 cases both died ; thus the chance of survival of the boy of such a pair is raised from 29 per cent. to 52 per cent. by the survival of his sister ; while the chance of survival of the girl is raised from 32 per cent. to 56 per cent. by the survival of her brother. Finally, of 140 pairs of two girls, in 42 cases both survived, in 53 cases only one survived, and in 45

cases neither survived ; consequently the chance of survival of a girl is raised from 37 per cent. to 61 per cent. by the survival of her sister. The association seems to be just as close between children of opposite sex as between children of the same sex, a fact which suggests that, with the high mortality experienced by triplet children, genetic similarity has little to do with the similarity of the chances of life.

### 3. *Average Size of Triplet Children.*

The children actually measured numbered 49 boys and 68 girls. The averages of the six measurements taken for each type of triplet births and for the aggregate of all types are given in Tables III and IV.

Table III.—Average Size of Boys.

Type.	Number of individuals.	Stature.	Stem.	Span.	Left cubit.	Head.	
						Length.	Breadth.
		mm.	mm.	mm.	mm.	mm.	mm.
3 boys	18	1102	624	1090	292.0	178.3	143.4
2 boys, 1 girl	16	1127	640	1128	304.5	179.3	140.8
1 boy, 2 girls	15	1092	624	1100	290.8	176.3	140.2
Aggregate	49	1107.0	629.2	1105.6	295.7	178.0	141.6

Table IV.—Average Size of Girls.

Type.	Number of individuals.	Stature.	Stem.	Span.	Left cubit.	Head.	
						Length.	Breadth.
		mm.	mm.	mm.	mm.	mm.	mm.
2 boys, 1 girl	8	1106	628	1084	292.9	170.5	138.3
1 boy, 2 girls	38	1078	610	1071	283.9	172.2	135.5
3 girls	22	1065	608	1060	278.5	168.5	136.6
Aggregate	68	1077.0	616.0	1069.1	283.3	170.8	136.2

It would be of great interest to compare the general averages with those of children by single births of the same age. The growth curve of children is, however, only known for a few special districts, and no general figure is available for the whole country, such as would be comparable with the triplet

material. Dunstan (2) gives valuable figures based on 300 measurements of stature in each trimester for the children of the rural area of E. Sussex. At  $6\frac{1}{2}$  years of age his boys averaged 1114·4 mm. and his girls 1103·2 mm. Comparison may also be made with the average heights obtained from Miss Elder-ton's distribution for Glasgow School children (1), Table III, p. 296.

Table V.—Average Stature of Children at  $6\frac{1}{2}$  Years.

	Triplets.	E. Sussex, rural.	Glasgow A, poorest district.	Glasgow B, poor district.	Glasgow C, better class.	Glasgow D, still higher class.
Boys	mm. 1107·0	mm. 1114·4	mm. 1070·6	mm. 1093·4	mm. 1093·4	mm. 1115·0
Girls	1077·0	1103·2	1065·6	1088·4	1087·2	1111·2

The differences between the different districts, though not exceeding 2 inches, are sufficiently great to leave in some uncertainty the comparison of triplet with single children. They are, of course, far too great to be ascribed to random sampling, but neither environmental nor hereditary factors can be excluded. With boys the average stature at this age in E. Sussex agrees with the "still higher class" schools in Glasgow, and only these two give a higher average than the triplets; the triplet girls do not compare so well, being taller only than the Glasgow girls from the lowest class districts. The averages cannot be taken to indicate that triplet children are on the average less tall at  $6\frac{1}{2}$  years of age than are children from single births. The distinguishing feature of the triplet measurements is not their average values, but the large sex-difference. In the collections based on large samples to which the comparison is made, the sex difference is about 11 mm. in E. Sussex and only about 5 mm. in all the Glasgow groups; the triplets show a difference of 30 mm. If standard errors are calculated for the triplet children regarded as independent samples, the values would be only 6 and 5 mm., and would not suffice to explain the large sex difference.

Actually, the children are drawn in batches of two or three from the same family, a circumstance which tends somewhat to increase the liability to error of the two averages; on the other hand, the fact that many of the pairs of opposite sex are brother and sister will tend to diminish the error of the difference. In order to obtain a strict test the sex difference was obtained for the 23 families in which both boys and girls had been measured, counting each family as only a single observation. The mean sex difference is then found to be 18 mm., with a standard error of nearly 9 mm.; there are, in fact, somewhat

large differences between different families measured in the relative stature of the boys and the girls, but the remarkable sex difference observed in the stature of the triplets is largely due to the boys having come predominantly from the taller families.

The average stature of the two sexes, 1092 mm., is seen to correspond with districts B and C of Glasgow, being about three-quarters of an inch less than that of rural E. Sussex and Glasgow district D, and nearly an inch greater than Glasgow district A. This simple result is one of the most surprising of the enquiry. It is well known that the correlations observed between the measurements of near relatives are only intelligible on the view that at least 90 per cent. of the variance observed in adult stature is due to genetic differences (8). This conclusion does not exclude the induction by environmental causes of an increase or decrease of stature of the order of half an inch, as a frequent occurrence; nor does it exclude effects of greater magnitude induced by extraordinary circumstances. The pre-natal conditions of triplet children must be exceptionally unfavourable, and the birth is very frequently premature. Perhaps the best measure of the severity of the environment which they have experienced consists in the very large post-natal death-rate. The conditions are sufficiently severe to kill more than half the children, but appear to be almost, if not entirely, inoperative in checking the normal growth of the survivors.

It will not be argued that the recovery of these children to normal stature is due to their environmental conditions during infancy and early childhood being in any way above the average; on the contrary, since the wage system in this country makes no allowance for the burden of offspring, we may be certain that the very existence of the triplet children will ordinarily induce a condition of economic hardship, which will last at least as long as the children remain dependents. An equally important circumstance is that, apart from economic stress, the amount of the care and attention ordinarily bestowed by their mothers upon children at these ages must be much diminished. It would appear that we must conclude that whatever may be the case in animals with a shorter growth period, the long duration of infancy and childhood in man gives ample opportunity for full recovery from the effects of pre-natal conditions, and for the full realisation of the genetic potentialities, even in characters, such as general growth, in which environmental modification would seem to be most readily effected.

#### *4. Precision of Measurements.*

The precision attainable in different human measurements does not seem to have been at all fully studied. In an investigation like the present in which it is important to determine with accuracy the degree of resemblance between children, many of whom may be of monozygotic origin, it is essential not only that no effort should be spared to attain adequate accuracy, but that objective evidence of the accuracy actually attained should be put on record. It was therefore arranged that for the majority of the cases measured, and especially for those of which the author had the greatest suspicion (namely, his own) that not only should the measurer satisfy himself that an accurate measure had been taken, but also that independent duplicate measures of the same value should be obtained, in order to yield by subsequent comparison an independent estimate of the accuracy of measurement. In doing this the specific purpose of the accuracy aimed at was held in view. The differences between different families of children are comparatively large, and these differences are to be used only in estimating the general variance of triplet children of a given age. The small systematic errors which undoubtedly exist between the measures obtained by different observers using different instruments will be entirely unimportant in estimating this variance, which will, on the contrary, be seriously affected by paucity of material, and should, in the author's opinion, be obtained from such an accurate estimate of the variance of the population as could be made from a carefully planned anthropometric survey based on the sampling method. In the second place, the small fluctuations, which, on the analogy of laboratory determinations would doubtless affect the same observer making the same measurements on different days, have fortunately no relevance to the comparisons between the measurements of different children on the same day and in the same circumstances. The errors in such comparisons should, in fact, be perfectly represented by the discrepancies between duplicate measurements taken at the same sitting.

The variance of a single observation of each measurement is given in Table VI.

If two values for the same measurement showed a marked discrepancy, a third was taken, a procedure which served to exclude gross errors. All measurements left on record were used in the above table, each case contributing 1 or 2 to the number of comparisons according as 2 or 3 measurements were left on record. The standard error of the values adopted will be nearly that calculated from the mean of two measurements, though this will be a slight over-estimate

Table VI.—Variation in Reported Measurements.

	Number of comparisons.	Single measurements.		Mean of 2, standard error.	Per cent. of like-sex variance.
		Variance.	Standard error.		
		mm <sup>2</sup> .	mm.	mm.	
Stature .....	77	6.171	2.48	1.89	0.57
Stem length .....	73	12.751	3.57	2.53	2.80
Span .....	76	40.964	6.40	4.53	3.15
Cubit .....	77	3.356	1.83	1.30	3.03
Head length .....	75	0.691	0.831	0.588	3.53
Head breadth .....	70	0.331	0.575	0.407	2.46

owing to the cases, which include measurements made in the least accurate circumstances, in which the mean of three values was employed. The last column expresses the variance ascribable to errors of measurement as a percentage of the variance ascribable to differences between triplets of like sex, calculated in a similar way. Since in no case this amounts to 4 per cent., it is evident that the estimate of degree of resemblance even between the like-sex pairs, will not be vitiated in the case of any one measurement by the working errors.

Dahlberg (3) gives a most valuable account of the errors of measurement detected in his study of the resemblance between twins. The values given, on p. 199 of his book, for 15 bodily measurements refer to the standard difference between single measurements, and are therefore double the standard errors of the mean of two measurements. For length of head, Dahlberg's figures indicate a higher precision than that attained in this enquiry, for the standard error of the mean of two of his measurements would be only 0.35; for breadth of head, his standard error, 0.415, is about the same as that given by our own data, but for stature his value is 2.69, a larger value than the 1.89 indicated above, though not one which would detract from the value of his observations.

### 5. Degrees of Resemblance.

5.1. *Resemblance of Triplets of Unlike Sex.*—The triplets living to be measured supply 34 cases of pairs of opposite sex. These must be regarded as dizygotic pairs. If they are also biovular, provided the fraction of the variance for which environmental variations is responsible is quite small, they should show the same degree of correlation as that shown by adult brothers and sisters by different births. This test was applied to Lauterbach's data, an attempt being

made to eliminate the age of measurement by fitting a cubic growth curve to the measurements of each sex. The values then found were somewhat low (see Table VIII) compared to well-ascertained fraternal correlations from single births, with the exception of that for cephalic index, which in that body of material showed no appreciable differentiation due to age or sex. It might therefore be supposed that this procedure could not, in view of the marked spurt of growth at puberty, entirely eliminate the disturbance due to varying ages.

As appears from the table, the present material based on measurements at a fixed age does, in fact, yield higher values, comparable with that obtained for cephalic index in Lauterbach's data. The natural supposition that weight, which yields the lowest unlike-sex correlation, is in reality not so highly correlated, even in growing children, as are the skeletal measurements, is not supported by the high values found for this variate between like sex pairs. The difference between the averages of the two series is, therefore, in so far as it is not ascribable to random sampling, probably due to the uniform age of the triplets.

Table VII.—Covariation of Pairs of Opposite Sex.

	Mean.		Sum of squares.		Sum of products.	Correlation.	
	Boys.	Girls.	Boys.	Girls.		r.	z.
Stature .	1104	1083	71,540	51,594	32,733	0.539	0.602
Stem length .	630	622	17,473	8,984	51,123	0.409	0.435
Span . .	1111	1073	86,927	62,219	37,946	0.516	0.571
Cubit .	296.4	286.5	12,206	8,926	7,032	0.645	0.767
Head length .	176.8	170.3	1,899.0	1,059.7	670.0	0.472	0.513
Head breadth . .	140.0	136.4	1,084.1	709.4	573.4	0.654	0.782

Table VIII.—Correlations between Brother and Sister.

		r.	Mean r.
Single births	Stature . . . . .	0.553	0.508 ± 0.012
	Span .. . . .	0.525	
	Cubit . . . . .	0.440	
Twins	Stature . . . . .	0.455	0.458 ± 0.053
	Stem length . . . . .	0.461	
	Weight . . . . .	0.380	
	Cephalic index . . . . .	0.537	
Triplets	Stature . . . . .	0.539	0.545 ± 0.074
	Stem length . . . . .	0.409	
	Span . . . . .	0.516	
	Cubit . . . . .	0.645	
	Head length . . . . .	0.472	
	Head breadth . . . . .	0.654	

In view of the sampling errors no significance should be attached to differences between the correlations of the different characters; comparison of the average correlations obtained from single births (7), twins (4), and triplets leaves no doubt that the correlations are substantially equal. Moreover, if any appreciable part of the correlation observed between brothers and sisters were not of genetic origin, but were due to the general similarity of the environment to which children of the same parents are exposed, the correlation should be appreciably higher with twins or triplets. Since this is not the case the data confirm the view that no appreciable proportion of the resemblance is due to similarity of environment.

It should be noted that the range of home environment in the case of triplets was extremely high. In not a few cases the support of the triplets, in addition to previous children, sufficed to lower the subsistence level to one of extreme hardship during the childhood of the triplets. Other cases were comparatively well-to-do. In somewhat less degree it is probable that the same is true of the American twins in Lauterbach's record. The point is one of some importance, since it may reasonably be argued that Pearson's data, predominantly from the families of University students, do not represent such extreme environments as would be sufficient to produce permanent retardation of growth; and that the conclusion drawn (8) by comparison of these values with expectation on the assumption of Mendelian inheritance, that no appreciable fraction of the variance can be ascribed to environmental differences, though true for the class sampled by Pearson, ought not to be extended to classes in which child life is attended by real and severe hardships. The data presented do not suggest appreciable environmental effects in any class for children born from 1917-1920; nevertheless, on a point of such importance it may be worth while to call attention to the possibility of making a crucial test of the matter with more precision than the present enquiry attempts, by consistently accumulating the evidence supplied by triplets, as in this country it is possible to do, taking as control an adequate number of children by single births of the same age, but of superior class.

5.2. *Resemblance between Triplets of Like Sex.*—The correlation between pairs of triplets of like sex may best be found by taking the mean measurements of two or more such children in the same way as have been treated the two or more measurements of a single child. In this way not only is the constant negative bias of intra-class correlations derived from a symmetrical table avoided, but no undue weighting is given to the sets of three as opposed to the sets of two; moreover, the accuracy of the determination corresponds to that of a number of simple pairs providing an equal number of comparisons. The



measurements comprise six cases of all three children of the same sex living to be measured, and 32 pairs of the same sex, the material being thus equivalent in respect of the estimation of the variance within such a trio to 44 pairs.

The value thus obtained, for the variance within a trio, is shown below (Table IX), for the six characters measured. It is interesting to compare them with the results obtained by Dahlberg (3). Dahlberg expresses his results as a mean difference between pairs of twins; his figures have therefore been squared and multiplied by  $\pi/4$ . Mean values for boys and girls have been used, and since Dahlberg's material consists of persons of very different ages, the comparison may best be made on the basis of the average actual measurement at each age. The unit is thus one-thousandth of the mean measurement in each case. Comparisons are possible for three characters. By a careful examination of the facial resemblance, and especially of the character of the ears, Dahlberg has diagnosed his material as monozygotic and dizygotic respectively, ignoring for this purpose the measurements actually obtained. The comparison thus provides a rough means of estimating what percentage of the triplets of like sex are of these two types of origin, assuming the accuracy and comparability of Dahlberg's figures.

Table IX.—Like-Sex Variance within Twinship or Trio.

	Triplets.	Dahlberg dizygotic.	Triplets.	Dahlberg monozygotic.	Triplets monozygotic.
	mm <sup>2</sup> .	per mille.	per mille.	per mille.	per cent.
Stature	541.7	914.6	454.2	100.3	56.5
Stem length	227.4				
Span	651.0				
Cubit	55.38				
Head length	9.789	647.8	321.8	157.9	66.5
Head breadth	6.729	557.0	348.7	113.5	47.0

The mean percentage monozygotic is about 57 by this method. This estimate agrees well with one based on the sex proportions of the group of triplets concerned, for of the 44 cases, 10 belong to sets of 3 boys, 6 to sets of 2 boys and 1 girl, 15 to sets of 1 boy and 2 girls, and 13 to sets of 3 girls. The number of cases in which all the children are of like sex is thus 23, against 21 of mixed sex. The proportion of monozygotic like-sex pairs may be estimated as  $3 \times 23 = 48$ , against  $2 \times 21 = 42$  dizygotic; a method of estimation which yields 53.3 per cent. monozygotic. Such a close agreement doubtless owes something to chance, while, on the other hand, it undoubtedly strengthens both estimates.

To obtain correlations between triplets of like-sex, comparable with those obtained for triplets of unlike sex, the individual variance for members of such pairs must be compared with the general variance of the population from which they were drawn. It was no part of the design of this research to obtain final figures for this variance, which, as was pointed out above, would be better based upon a comprehensive anthropometric survey; the data do, however, supply a tolerable basis for estimation, in the variance found among all children of like-sex who were measured. There were of these 49 boys and 68 girls. Since the girls also preponderate (28 pairs to 16) in the like-sex pairs, the variance obtained from these two groups may be pooled as they stand, giving an estimated variance with precision appropriate to 115 degrees of freedom. This does not preclude large sampling errors, but the results can be little affected at most by any real difference in variability which may exist between the two sexes. The like-sex correlation is then found by subtracting from unity the ratio of the individual variance of a member of a like-sex pair to the individual variance of a member of the general population.

The correlations, thus obtained between triplets of like sex agree closely with those obtained by the author for the like-sex twins of Lauterbach's data.

Table X.—Correlations between Pairs of Like-Sex.

Triplets.			Twins (Lauterbach).		
	<i>r.</i>	<i>z.</i>		<i>r.</i>	<i>z.</i>
Stature . . . . .	0·720	0·908	Stature . . . . .	0·735	0·940
Stem . . . . .	0·498	0·547	Stem . . . . .	0·575	0·655
Span . . . . .	0·735	0·938	Weight . . . . .	0·735	*0·938
Cubit . . . . .	0·803	1·107	Cephalic index .	0·731	0·931
Head length . . . .	0·766	1·010			
Head breadth . . . .	0·764	1·006			
Mean . . . . .	0·726	0·914	Mean . . . . .	0·699	0·866

In both series stem length shows a surprising departure in the direction of low correlation. In Lauterbach's data the effect could merely be noted as an anomaly; in the triplet data, though not in the twin data, it is also the lowest correlation for children of unlike sex. It was noticed early in the measurements that the values obtained for stem length were very sensitive to the posture in which the child was placed. Care was therefore taken to eliminate as far as possible such variations; the children were seated on a firm horizontal surface, usually a kitchen table, with their knees projecting as little as possible beyond the edge. I have, however, still suspected that the clothing, and especially the

habitual sitting posture, of the child might considerably affect the measurements. In view of the slight discrepancies between duplicate measurements taken at about half an hour's interval, which only account for a depression of the value of the correlation by 0.014, the conclusion that difficulties of measurement are alone responsible cannot be regarded as established, and it may be that this measurement, unlike others, admits, at least in the growing child, an appreciable amount of environmental modification. Such a view is not free from difficulty, in view of the fact that with pairs of unlike sex the triplet data show little, and Lauterbach's data no effect, and, in addition, the effect with twins of like sex in Lauterbach's data is much more pronounced with boys than with girls. The discrepancies obtained from Lauterbach's measurements and my own should in any case be sufficient to put investigators on their guard against basing important conclusions on this particular character, without a thorough scrutiny of the reliability of the measurement technique to be employed.

If stem length is set aside as of doubtful reliability, the mean correlation from the triplets, obtained as usual from the values of  $z$  ( $=\tanh^{-1}r$ ), is 0.759, while that from Lauterbach's twins is 0.734. Neither with nor without stem length are the values significantly different. This agreement supplies a further confirmation of the estimates made above of the proportion of monozygotic pairs, for this proportion was estimated in Lauterbach's data to be about 59 per cent.

Further and concordant evidence of the proportion of pairs to be regarded as monozygotic may be obtained from the values of the correlations themselves. Even if it were assumed that monozygotic twins were identical in their physical measurements, then if the dizygotic like-sex pairs were no more highly correlated than pairs of unlike-sex, it would require 47 per cent. monozygotic to raise the correlation from 0.545 to 0.759. This percentage may therefore be regarded as a minimum. If, on the contrary, we take the proportion as estimated by comparison with Dahlberg's data for the relative variance, and from the sex distribution (namely, 57 per cent.), then the correlation between monozygotic twins should be 0.920 in order to reproduce our correlations. A similar estimate made from Lauterbach's data gave values between 0.92 and 0.93 for all three characters other than stem length. In the values from the triplet data also we have omitted stem length from the average, and have also, on the other hand, made no allowance for the known errors of measurement which will have depressed all the other correlations by about 0.008.

Since an intra-class correlation measures the fraction of the variance ascribable to differences between the classes compared, a correlation of 0.93 between mono-

zygotic twins may be interpreted as meaning that 93 per cent. of the human variance in physical measurements is to be ascribed to hereditary factors, leaving 7 per cent. for other causes of variation. Exactly what these other causes of variation are, it is extremely difficult to indicate. Gross factors, such as the supply of nutrients, facilities for exercise, and all other conditions open to inspection and regulation, may have very little influence; these would certainly cause smaller variations between twins than between unrelated children. Other causes, such as the onset, and age of onset, of infectious diseases, depend from less obvious environmental differences, and their effects will in consequence have a more accidental character. If, as seems probable from the mutual interaction of the parts in development, very minute initial variations of growth of particular organs may in some cases lead to appreciable ultimate difference in the measurements, these will be as great for twins as for unrelated children. Indeed it may be suspected that they will be greater, for the great differences in weight at birth show that the pre-natal condition of children born together must in some respects be strikingly different.

An exact estimate of the average correlation in metrical characters between identical twins should evidently combine a critical diagnosis based upon Dahlberg's methods, carried out independently by two different judges, without knowledge of the measurements; an equally necessary factor in such an estimation, and one which is unfortunately wanting from Dahlberg's data, is the value of the variance at a given age of the general population sampled. Experts differ profoundly as to the correct diagnosis of monozygotic and dizygotic twins (11, 12); nevertheless, the concordant results obtained by comparing the triplet data with Dahlberg's averages have convinced the writer that his method of diagnosis, which was published during the progress of the triplet enquiry, has gone far to solve the problem of discriminating the two types of twins. Such discrimination can, however, never be absolute, if only because genetic identity itself is not impossible for dizygotic twins.

The question whether all three triplet children can ever be regarded as monozygotic should evidently be answered confidently only on a larger basis of observation than the six cases for which measurements were obtained. A rough criterion based on the measurements may be made by excluding all cases which differed in any measurement by more than a fixed maximum difference. Taking arbitrary standards proportional to the standard differences between like-sex pairs, namely, for stature 40 mm., for stem length 26 mm., for span 44 mm., for cubit 13 mm., for head length 5.4 mm., and for head breadth 4.5 mm., only 22 pairs remain sufficiently alike in all measurements. These will doubtless

be predominantly monozygotic pairs, but the criterion is far from decisive, and some overlap will doubtless have occurred. In no case do all three triplets fulfil the conditions. In three cases, numbers 1, 59 and 61, one child is considerably larger than the other two. In cases number 1 and 61 the two smaller children are alike in their measurements, in case number 59 they differ by 8 mm. in head breadth; this case is probably trizygotic. In the three other cases monozygotism cannot be confidently denied. In case 52, (c) is in most respects the smallest child, but only differs distinctly from her sisters in being shorter (by 5·6 and 7·2 mm.) in the head. In case 2 all three girls agree closely save that (c) is narrower (by 5 and 7 mm.) in the head. In case 40, (a) is both shorter in sitting height and narrower in the head than her two sisters. Since the standard difference in head length between monozygotic pairs, as judged either from Dahlberg's data or from the triplet measurements, cannot differ much from 3·1 mm., the case for regarding case 52 as monozygotic is particularly strong.

#### *6. Inheritance of Tendencies favouring Multiple Births.*

Much confused controversy has turned upon the inheritance of tendencies favouring twin births, and especially upon the influence of the father upon the production of twins. The most self-consistent view seems to be that developed by Weinberg, namely, that there is no paternal influence, and that the production of monozygotic twins is not inherited. This view is not only self-consistent but is supported by a considerable quantity of genealogical statistics; it appears indeed, without critical scrutiny, to be the only view consistent with the figures which Weinberg presents. Unfortunately, it has not been borne out by any other substantial body of data. The situation involves a definite conflict of evidence, and it is undesirable that this prime fact should be obscured, although it must necessarily be complicated by the conflict of inferences drawn from the evidence. The best hope of progress evidently lies in obtaining data from entirely fresh sources, taking every care to eliminate those causes of error which seem most likely to have produced the existing discrepancies.

A further point of importance is that conclusions should be based whenever possible upon comparisons made within a homogeneous body of data, rather than with data obtained by a different method, for apart from the potent influence of unconscious selection, differences in the completeness with which cases of twinning involving one or more still-births are recorded will much affect the frequency of twinning observed. That this precaution is not unnecessary may be seen from the great differences in absolute frequencies obtained by different observers in districts in which the official statistics show no comparable discrepancies.

The triplet data were entirely unselected save for the fact of the occurrence of triplets. Of the 148 families asked for information concerning related births, 98 provided complete and satisfactory information. It will be preferable to use only this unquestionable portion of the data; only one case of those omitted need be mentioned separately, namely, a family of 11 births, of which 2 were of triplets and 5 of twins, yielding in all 20 children. The inclusion of the case would evidently have much raised the observed percentage of twins in the families containing triplets, and possibly also in related families. Its omission, however, cannot invalidate the conclusions to be drawn from unquestionably correct information, for it is omitted not because the information was exceptional in character but because it was confused and very incomplete. A number of such cases of exceptional fecundity have been reported from time to time, and may represent a phenomenon distinct from the normal, though rare and scattered, occurrence of multiple births.

Full data for related multiple births are shown in Tables XI, XII and XIII. Among the families in which the triplets occurred, 493 other births are recorded, of which 14 were of twins and 4 of triplets. Counting triplets as equivalent to the production of twins twice (for, in addition to the production of twins, a second fission or additional ovulation must have occurred), the percentage of twinning in these families is 4·5 per cent. The ratio of triplets to twins is high, but this may be no more than a coincidence. It is to be presumed that the proportion of twins in Great Britain and Ireland is not greatly different from 1 per cent., and the other data of this enquiry show that it cannot be much more. Consequently a rate of 4·5 per cent. represents a very considerable concentration of multiple births, and shows that whether the tendency is inherited or not, certain parents are definitely characterised by higher twinning rates than are others.

Table XI.—Other Children of Parents.

Type of triplets.	Families.	Single births.		Twins.			Triplets.			
		Boys.	Girls.	2 boys.	1 boy, 1 girl.	2 girls.	3 boys.	2 boys, 1 girl.	1 boy, 2 girls.	3 girls.
3 boys	13	33	29	—	4	1	—	—	—	—
2 boys, 1 girl	27	79	73	—	1	—	—	—	1	1
1 boy, 2 girls	39	82	81	2	3	1	—	1	—	—
3 girls	21	45	53	1	1	—	—	1	—	—
Total	100	239	236	3	9	2	—	2	1	1
		475		14			4			

Table XII.—Children of Mother's Parents.

Type of triplets.	Families.	Single births.		Twins.			Triplets.
		Boys.	Girls.	2 boys.	1 boy, 1 girl.	2 girls.	3 boys.
3 boys	13	45	48	1	3	—	—
2 boys, 1 girl	27	80	102	—	1	2	1
1 boy, 2 girls	39	125	161	3	4	1	—
3 girls	21	76	89	—	1	2	—
Total	100	326	400	4	9	5	1
		726		18			

Table XIII.—Children of Father's Parents.

Types of triplets.	Families.	Single births.		Twins.			Triplets.
		Boys.	Girls.	2 boys.	1 boy, 1 girl.	2 girls.	
3 boys	13	62	41	1	—	—	—
2 boys, 1 girl	27	114	85	3	—	2	—
1 boy, 2 girls	39	151	120	3	1	2	—
3 girls	21	85	59	1	1	1	—
Total	100	412	305	8	2	5	0
		717		15			

Evidence of heredity must, of course, be drawn from the corresponding data for related families. Of 745 births to parents of the mother, 18 were of twins and 1 was of triplets; calculated as before, this gives 2·7 per cent. twins in these families. Similarly of 732 births to parents of the father, 15 were of twins; this is 2·0 per cent. The statistical significance of both results may be gauged by the fact that in a Poisson series with mean 7·3 a value as high as 15 will only occur eight times in a thousand trials.

The data for the families of the parents of triplets thus give definite support to the view that the production of multiple births is influenced to an important extent by the hereditary qualities of the father. Of the similar evidence from the relatives of the parents of twins, perhaps the most comparable is that given by Danforth (6) from St. Louis. It is stated that "the investigator had no knowledge of any of these families until in each case the birth of twins was

reported to the bureau of vital statistics." Fifty families were investigated; of 181 births in the same families 10 were of twins (5·5 per cent.), of 328 births to parents of the mothers 10 were of twins (3·0 per cent.), while of 297 births to parents of the father 8 were of twins (2·7 per cent.). The twin data are smaller in volume, being based upon 50 families against 98 available for the triplet enquiry; the twinning percentages are distinctly higher in each case, although the families were selected merely by a single occurrence of twins, instead of by the occurrence of triplets; but the main conclusion that the selected families show the highest twin frequency, and that their relations on both sides show the same effect to a diluted extent, appears clearly from both enquiries.

Davenport (5) gives figures for a somewhat more highly selected group of families who have had twins twice. Of 355 births to the parents of twin-repeating mothers 16, or 4·5 per cent., were of twins; of 289 births to the parents of twin-repeating fathers 12, or 4·2 per cent., were of twins. The discrepancy in the actual percentages is here more striking than in those of Danforth, and we have in this case no assurance that cases of special interest do not find their way, more readily than cases showing no heredity, into the archives of the Eugenics Record Office. That a somewhat severe concentration of interesting cases may have occurred in this material is suggested by the percentage of twin births obtained for the brothers and sisters of repeating fathers and mothers. These are father's sisters' children, 8·2 per cent.; mother's sisters' children, 5·5 per cent.; father's brothers' children, 6·5 per cent.; mother's brothers' children, 4·5 per cent.

These high percentages, while apparently supporting, in reality introduce a difficulty to the theory developed by Davenport, for he infers from their equal paternal and maternal influence. Now if we select a person specially characterised, such as a twin-repeating father, or a twin-repeating mother, we may infer on this theory that both the parents of this person will (in less degree) be similarly characterised, and the children of the marriage between two such parents will therefore be twins with nearly double the frequency which would have been realised if only one of the parents had been so characterised; although, therefore, a brother or sister is genetically as close a relative as a father or mother, the theory is not compatible with the view that the sister or brother, married to some quite unselected person, will have twins as frequently as will the father and mother married to each other.

If 6 per cent. twins is correct for the brothers and sisters of the chosen parents, we might reasonably expect 11 per cent. twins from their fathers and mothers; on the other hand, if 4·4 per cent. is correct for the fathers and mothers, we



might reasonably estimate the percentage for the brothers and sisters at 2·7 per cent. If heredity is at work it should, in fact, be made manifest not only by the contrast between the selected families and the general population, but equally by the contrast between different degrees of affinity to the *præpositus*, or selected centre, of each pedigree. The latter type of contrast is indeed the more important, since, as in England, the general population may be almost unknown, and, in other cases, as the divergences between different enquiries indicate, discrepancies of unknown origin have certainly been introduced between the official and the genealogical data; consequently, in view of these, the comparison of different groups of relatives in the same material provides undoubtedly the best controlled tests available of the hypotheses to be considered.

One probable reason why this contrast, to be expected between different groups of relatives, has not been realised, or at least utilised, lies in the fact that the groups of relatives appropriate for comparison will be different, according to whether paternal influence is admitted or not. If paternal influence were absent, not only would the relatives of the father and the mother's brothers show no excess of twins, but the sisters and daughters of the mother should show no less effect than the mother's mother. This contrast between the twin-frequency of the mother's sisters on the one hand with the mother's brothers, the father's sisters and the father's brothers on the other, seems to provide a well-controlled method of determining if any important group of the causes of multiple births are limited, as Weinberg suggests, to action through the mother. Equally, however, the contrast between the twin-frequency exhibited by the parents and that of the brothers and sisters must be manifest if to any important extent twinning is inherited through both parents alike. Neither contrast appears in Davenport's figures in spite of the high percentages of twinning observed, a circumstance which makes it impossible to use these results with confidence as a means of discriminating between the two opposed views.

Much lower percentages of twins are found among the relatives of twin-bearing parents by Weinberg and Dahlberg. From Wurtemberg Weinberg finds 101 fathers of twins from families with 901 children in all, among whom 6 pairs are twins, thus showing no indication of paternal influence. More extensive data for the relatives of mothers are given for Stuttgart (3, p. 107).

Table XIV.—Related Births to Mothers of Twins.

	Relatives of mothers of twins of like sex.			Relatives of mothers of twins of unlike sex.		
	Mother.	Sister.	Daughter.	Mother.	Sister.	Daughter.
Multiple births . . . . .	61	55	44	45	24	27
Single births . . . . .	3909	2524	3386	1803	998	1437
Total . . . . .	3970	2579	3430	1848	1022	1464
Percentage . . . . .	1·53	2·13	1·28	2·50	2·35	1·84

The percentage of twinning is seen to be much lower than in either of the American groups of data discussed, that for the mothers being more comparable with the values obtained from the triplet data. For each degree of relationship the relations of twins of unlike sex have the higher percentage, and Weinberg concludes that only the tendency to dizygotism is inherited, that for diembryony showing no hereditary tendency. It should be noted that in Weinberg's material this difference is only statistically significant in the case of the mothers, though the daughters, and even to a very slight extent the sisters, show a tendency in the same direction. If the above figures are examined critically, it appears that the three classes of relatives do not give, within the limits of reasonable sampling errors, the same percentage of twins. This effect seems to be wholly due to the larger material on the relations of twins of like sex. In these the sisters show much the higher percentage. It is possible to give a plausible, though tentative, explanation of these discrepancies.'

Dahlberg (3, p. 113) has criticised the material on the ground that incompleteness of registration may have considerably reduced the twin percentage. If this is so the sisters, which show the highest twin frequency, would seem to represent the generation in which the data are most complete, and it constitutes a serious *caveat* against Weinberg's conclusions—that all hereditary effects should be ascribed to dizygotism—that it is precisely in the sisters that no appreciable difference exists between the relations of twins of like and of unlike sex. Dahlberg has shown (3, p. 47) that the omission of still-births diminishes especially the percentage of monozygotic twins.

Upon the question of the inheritance of diembryony the triplet data appear to supply evidence of a decisive character. For of the 15 twin births of the parents of the father of the triplets only 2 are of opposite sex, whereas of the 18 twin births of parents of the mother 9 are of opposite sex. The whole

of the twins, in excess of expectation for ordinary families, born to the parents of the fathers, are thus of like sex, and presumably therefore all monozygotic. It is not that every one of the 13 pairs found need be monozygotic, for two or three like-sex dizygotic pairs might well occur in 700 births taken at random. That 13 such pairs should occur is beyond reasonable probability, and since there is no evidence of any excess of dizygotic pairs, the plain inference from the data is that the paternal influence upon twinning is confined to the production of diembryony. Since in such cases fission occurs after the union with the egg of the paternal gamete, it is only natural that for this process developmental tendencies inherited from the father should be of equal importance with those from the mother. Whether the tendency to diembryony is also inherited through the mother, as seems *a priori* probable, could only be decided upon larger data, for the presence of an excess of dizygotic twins among the relations of the mother must much increase the sampling errors of any estimate of diembryony.

It is to be noted that all the groups of relationship data cited agree in giving a slightly higher twin percentage to the relations of the mother, though in no case, save that of Weinberg, is this excess statistically significant. It does, however, accord with the view that, while paternal influence only affects the one type of twinning, maternal influence may act upon both. If, further, it is assumed that the maternal influence in diembryony is equally potent with that of the father, the smallness of the differences usually found between the relations of the two parents makes it probable that heredity is less influential in inducing dizygotism than it is in inducing diembryony.

This view is the direct contrary of that held by Weinberg; on the other hand, it tallies with Davenport's finding even higher twin-frequencies among the relatives of monozygotic twins than among those of twins in general. It may, in addition, be supported by facts other than the twin-frequency of relatives. It is well known that the percentage of twins increases greatly with the age of the mother. Knibbs (9, p. 309) shows a threefold increase between 20 and 38. Comparisons of the sex distribution at each age, of which the most thorough is that of Dahlberg (3, pp. 21-28), shows that this increase is certainly largely and probably wholly due to a change in the frequency of dizygotic pairs. Evidently, then, dizygotism is much influenced by an environmental factor, age of mother, upon which hereditary influences can have but little effect. Diembryony, on the other hand, is apparently not influenced at all by this factor. In consequence, it is possible to hold that the probability of monozygotic twins depends wholly upon the hereditary nature of the parents, while in the case of

dizygotism any evidence for heredity is bound to be much obscured by the mere effect of variations in the age at childbirth.

If a strongly heritable maternal tendency towards dizygotic twinning existed, it would appear in contrasting the twin-frequency of mother's sisters with those of mother's brothers, father's brothers and father's sisters. No such effect appears in Davenport's data, and the comparison cannot be made in Weinberg's. The triplet data are as follows :—

Table XV.—Frequency of Multiple Births born to Brothers and Sisters of the Parents of Triplets.

	Mother's sisters.	Mother's brothers.	Father's sisters.	Father's brothers.
Single . . . . .	504	501	626	532
Twin . . . . .	5	6	7	7
Triplet . . . . .	1	—	1	—
Total . . . . .	510	507	634	539
Percentage . . . . .	1.4	1.2	1.4	1.3

No significant differences exist between the four classes, and it may be doubted if any of them differ significantly from the twin percentage of the general population. For this reason the sex distribution of the related twins is of no special interest. The actual types are, however, given below :—

Table XVI.—Sex Distribution of Related Multiple Births.

	2 boys.	1 boy, 1 girl.	2 girls.	3 boys.	2 boys, 1 girl.
Mother's sisters . . . . .	—	2	3	—	1
Mother's brothers . . . . .	2	1	3	—	—
Father's sisters . . . . .	1	3	3	1	—
Father's brothers . . . . .	—	4	3	—	—
Total . . . . .	3	10	12	1	1

Among the relatives of triplets the frequency of triplets compared to that of twins is somewhat high. Among children of the same parents we have 4 triplets to 14 twins, among the children of their parents, 1 triplet set to 33 pairs of twins, and among the children of their brothers and sisters 2 sets of triplets to 25 pairs of twins. The numbers are too small to give more than an indication, but they suggest that in addition to the factors favouring multiple births in general,

there may be others which favour the particular sequence of events, such as double ovulation followed by fission of one zygote, which gives rise to triplets.

It will be noticed that a perfectly clear contrast exists between the frequency of twins born to mothers (2·8 per cent.) and that born to sisters (1·4 per cent.) of mothers of triplets, in accordance with the other clear evidence which these data provide of paternal influence. . There is, however, one factor which should be mentioned, not as probably producing the whole effect, but as possibly contributing to it. The strong age association of dizygotic twinning renders the frequency of this phenomenon open to interference by artificial contraceptive methods. If these are employed after the production of a few children, a proportion of the married women concerned will only be "exposed to risk" during the earlier parts of the reproductive period. Such a tendency would certainly reduce the proportion of dizygotic, though probably not of monozygotic twins, and it is only in dizygotic twins that any excess is to be looked for of the mother's sisters against the mother's brothers, and the father's brothers and sisters. If, as there is ample reason to believe, contraceptive practices were more widely employed in the generation represented by mother's sisters than in the previous generation of mothers' mothers, we might reasonably put down some part of the contrast to this cause. It should perhaps be mentioned in this connection that the frequency of triplets, or at least of applications for the King's Bounty, has fallen off in the last 25 years more rapidly than would be expected from the decrease in the annual number of single births registered.

### *7. Summary.*

Measurements taken at a fixed age of 115 surviving triplet children show no distinct difference in stature from children by single birth. The precision of the comparison is, however, limited by our scanty knowledge of the average bodily growth in the general population of Great Britain and Ireland.

The average degree of resemblance between triplet children of opposite sex does not differ appreciably from that of twins of opposite sex, or of fully grown brothers and sisters by different births.

The differences between triplets of like sex are intermediate in magnitude between those found by Dahlberg in groups of like-sex twins classified as monozygotic and dizygotic respectively. The comparison indicates that about 54 per cent. of the like-sex triplets pairs measured were monozygotic. The correlation between triplet children of like sex, the estimate of which could be improved by a better knowledge of the general population, agrees well with that

found for like-sex twins in Lauterbach's measurements, and indicates a correlation between monzygotic pairs of about 0.92.

The material is not sufficient to establish definitely that in any set all three triplets were monozygotic, though this is a probable explanation of the resemblance in at least one set. The discrepancies between the data provided by different investigators upon the inheritance of tendencies favouring multiple births have prevented any substantial agreement on this subject. An examination of the material suggests that these discrepancies are due (a) to the accumulation of interesting cases showing apparent heredity in pedigree collections; (b) inequality in the completeness with which still-births are recorded in the different sources of material compared.

To avoid these discrepancies it is important to spare no pains to ensure that a strictly valid statistical technique is employed in the collection of data, a consideration which is of equal importance in the statistical study of rare occurrences of all kinds; and to utilise, as the best controlled evidence of inheritance, the contrast in twin-frequency between different groups of relatives in the same collection. When allowance is made for these two sources of discrepancy, existing data upon twins may be brought into harmony with the results of the triplet enquiry.

The triplet pedigrees show a significant excess of multiple births in the families of both the father and the mother of the triplets. In the case of the father the excess consists wholly of like-sex twins. In other material the sex distribution of the related twins seems not to have been recorded, in spite of evident importance for the classification of heritable tendencies affecting dizygotism and diembryony respectively. The triplet data indicate that the paternal influence is only exerted in the production of diembryony. It is probable *a priori* that the heritable constitution of the mother exerts an equal influence in this respect, but more extensive data would be required to demonstrate this.

Since, in these and other data, the maternal inheritance is not much stronger than the paternal, it is probable that dizygotism is less strongly inherited than is diembryony. This view is supported by the well-established increase in multiple births with increasing age of mother, which effect appears to be confined to dizygotism.

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## THE ESTIMATION OF LINKAGE FROM THE OFFSPRING OF SELFED HETEROZYGOTES.

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(With Two Text-figures.)

### 1. INTRODUCTION.

THE method of measuring the linkage between two factors by crossing the double heterozygote back to the double recessive, supplies a direct measure of the proportion of the "recombination" classes to the total count; no statistical methods are necessary in examining the data beyond expressing the number of offspring in the two recombination classes as a percentage of the total offspring counted.

It is frequently preferable, in view of the labour saved, to obtain a progeny by selfing the heterozygote. In such cases unlinked factors should give four classes of offspring in the ratio 9 : 3 : 3 : 1, and linkage will manifest itself by an excess of the first and last classes, if both dominant genes came from the same grandparents (Coupling), and by an excess of the second and third classes if they came from different grandparents (Repulsion).

It should be noticed that progenies of this kind are influenced by linkage both in the male and in the female gametogenesis. If therefore the recombination percentages are different in the two sexes, or if it is desired to study such differences as may exist, the method of back-crossing offers a considerable advantage. The method of selfing is capable of detecting such a sexual difference, as will be shown more clearly below, but is relatively insensitive for this purpose. If on the contrary, as is more usually the case, the object is to detect any linkage which may exist, the method of selfing is not in itself unsuitable. Its advantages would be more obvious if suitable methods had from the first been used in estimating the linkage value, for the method has suffered some unmerited disrepute from the extremely inefficient methods which have been usually employed in this process of estimation.

### 2. THE QUANTITATIVE EFFECTS OF LINKAGE.

A heterozygote **AaBb** of two factors **A** and **B** will produce gametes of four types **AB**, **Ab**, **aB** and **ab**. If the factors are unlinked these will be produced in equal numbers; if linked, the classes **AB** and **ab**

will appear with a frequency different from the classes **Ab** and **aB**. In general, whatever the origin of the heterozygote may have been, we may represent by  $q$  the proportion of the gametes belonging to the two latter classes, and by  $p$  ( $= 1 - q$ ) the proportion belonging to the two former. The same will be true both in male and in female gametogenesis, but since the value of  $p$  may be different for the two sexes, we may use  $p$  for the proportion in the female gametogenesis, and  $p'$  for the corresponding proportion in the male gametogenesis.

We shall then have gametes capable of mutual fertilisation produced in the following proportions:

	<b>AB</b>	<b>Ab</b>	<b>aB</b>	<b>ab</b>	
Ovules	$\frac{1}{2}p$	$\frac{1}{2}q$	$\frac{1}{2}q$	$\frac{1}{2}p$	
Pollen	$\frac{1}{2}p'$	$\frac{1}{2}q'$	$\frac{1}{2}q'$	$\frac{1}{2}p'$	.

It is evident that the fraction of offspring in the double recessive class is  $\frac{1}{4}pp'$ , and since this with either of the two singly recessive classes must make up a quarter of the whole, these must each have a fraction  $\frac{1}{4}(1 - pp')$ , leaving for the class which is recessive in neither factor, the balance of  $\frac{1}{4}(2 + pp')$ . The whole effect of linkage is therefore expressible in terms of the single quantity  $pp'$  which we may hereafter designate by  $x$ . If the recombination fraction is the same in both sexes, it will be obtained as the square root of  $x$  for repulsion, and as  $1 - \sqrt{x}$  for coupling.

If the value of  $\sqrt{x}$  for repulsion progenies differs significantly from  $1 - \sqrt{x}$  for coupling progenies, then the recombination values for the two sexes will be significantly different; they may be found by solving the equations

$$pp' = x_1,$$

$$(1 - p)(1 - p') = x_2,$$

which lead to a quadratic equation of which the roots are  $p$  and  $p'$ . The method as mentioned above is insensitive compared with the use of back-cross data, and when the two recombination values are found it does not tell us which belongs to which sex.

### 3. THE ESTIMATION OF LINKAGE.

The values of  $x$  may be estimated, from the observed numbers in the four classes, in a considerable variety of ways. We shall discuss the advantages enjoyed by some estimates over others derived from the same data. It will be made clear that some of the advantages are universal and of such a kind as we should always wish to obtain, others are adventitious and are only realised in special circumstances. In particular

the advantage of the class of estimates technically called *efficient statistics* over all other estimates is universal, while among the efficient group we may find some better than others in special cases. The comparisons may be conveniently made on a numerical example (Carver<sup>(7)</sup>), showing linkage between the sugary factor in maize and a factor for white base leaf. The case was one of repulsion, and the numbers of seedlings counted were

Starchy		Sugary		Total
Green	White	Green	White	
1997	906	904	32	3839

As a representative group of methods we may take the following:

(a) Additive method, also called Emerson's formula;  $x$  is calculated from the sum of the first and fourth classes.

(b) Weighted mean method;  $x$  is calculated from an alternative linear function of the frequencies.

(c) Product method;  $x$  is calculated from the products of the first and fourth classes and of the second and third classes; equivalent to the method of Bridges<sup>(2)</sup>.

(d) Method of maximum likelihood, first applied to this problem by Haldane<sup>(1)</sup> who derives a process of approximation which can only lead to this solution, from the condition that  $\chi^2$  shall be a minimum.

(e) Method of minimum  $\chi^2$ .

The estimate obtained by each of these methods is made to conform to the criterion of consistency, which simply involves the condition that if the sample of observations were increased without limit, the method would give the correct value to any required degree of approximation. This may be ensured by writing, instead of the observed frequencies, quantities in the theoretical ratio  $2 + x : 1 - x : 1 - x : x$  which will be approached as the sample is increased indefinitely. If then the observed frequencies are  $a$ ,  $b$ ,  $c$  and  $d$  in a total of  $n$  offspring, the first method

(a) will consist in equating  $a + d$  to its expected value  $\frac{n}{4}(2 + 2x)$ ; the resulting equation may be written

$$nx = a - b - c + d,$$

from which  $x$ , and thence the recombination percentage, may be immediately calculated. In our example we have

$$\begin{aligned} 3839x &= 219, \\ x &= .057046, \end{aligned}$$

giving a recombination percentage of 23.88.

For this estimate the comparison of expected with observed frequencies is as follows:

	Starchy		Sugary		Total
	Green	White	Green	White	
Expected ( <i>m</i> )	1974.25	905	905	54.75	3839
Observed	1997	906	904	32	3839
Difference ( $\delta$ )	+22.75	+1.0	-1.0	-22.75	0
$\delta^2/m$	0.262	0.001	0.001	9.453	9.717

The value of the measure of discrepancy  $\chi^2$  is 9.717 and for two degrees of freedom this will occur by chance less than once to each 100 trials. The conclusion indicated is that the number of sugary-white seedlings has been depressed by some cause additional to linkage. It will be shown that this conclusion is unwarranted, although it follows inevitably if this method of estimation is employed.

For each method of estimation it is possible to calculate the variance due to errors of random sampling; since the variance is not always the same for different methods of estimating the same quantity, these calculations have an important bearing upon the adequacy of any particular method in making use of the information supplied by the data. The variance of our estimate of *x* by method (*a*) is

$$\frac{1 - x^2}{n} \dots\dots\dots(1),$$

giving a standard error for *x* of .01601, or of 3.373 per cent. in the recombination percentage.

For method (*b*) we shall take the expression *a* - 3*b* - 3*c* + 9*d*, which, by the criterion of consistency, must be equated to *n* (4*x* - 1). In fact, the equation for *x* is

$$4nx = 2a - 2b - 2c + 10d,$$

or, numerically  $3839x = 173.5,$

$$x = .045194,$$

giving a recombination percentage of 21.26.

The comparison of expected with observed frequencies is now

	Starchy		Sugary		Total
	Green	White	Green	White	
Expected ( <i>m</i> )	1962.875	916.375	916.375	43.375	3839
Observed	1997	906	904	32	3839
Difference ( $\delta$ )	+34.125	-10.375	-12.375	-11.375	0
$\delta^2/m$	0.593	0.117	0.167	2.983	3.860

The measure of discrepancy found in this case is only 3.860, a value which will be exceeded by chance in more than 15 per cent. of trials.

This value therefore indicates that there is no significant departure from the expected frequencies, contrary to the conclusion drawn from method (a).

The random sampling variance of the estimate of  $x$  by method (b) is

$$\frac{1 + 6x - 4x^2}{4n} \dots\dots\dots(2),$$

giving a standard error for  $x$  of .00907, or of 2.133 for the recombination percentage.

It will be noticed that the standard error of the estimate by method (a) is materially larger than that by method (b). The latter method therefore makes use of a larger fraction of the information supplied by the data, and gives in consequence a better estimate of linkage.

In method (c) we have the equation for  $x$

$$\frac{x(2+x)}{(1-x)^2} = \frac{32 \times 1997}{906 \times 904},$$

a quadratic equation of which the positive solution is

$$x = .035645.$$

The comparison of expected with observed frequencies becomes

	Starchy		Sugary		Total
	Green	White	Green	White	
Expected ( $m$ )	1953.711	925.539	925.539	34.211	3839
Observed	1997	906	904	32	3839
Difference ( $\delta$ )	+43.289	-19.539	-21.539	-2.211	0
$\delta^2/m$	0.9592	0.4125	0.5012	0.1429	2.0158

The measure of discrepancy has fallen still further, and is now scarcely greater than its average value 2.0; the apparent defect of the double recessive class was evidently due wholly to the method used to estimate linkage.

The random sampling variance of  $x$  by method (c) is found to be

$$\frac{2x(1-x)(2+x)}{n(1+2x)} \dots\dots\dots(3),$$

giving a standard error for  $x$  of .00584, or of 1.545 for the recombination value.

The formula (3) is of special interest, for we shall find exactly the same expression for the random sampling variance of the two remaining methods of selection, namely the method of maximum likelihood and the method of minimum  $\chi^2$ . Now it has been proved(3) that the method of maximum likelihood will in all cases supply a solution of which the

random sampling variance is as small as possible; consequently the group of solutions which have the same random sampling variance is of special importance in that they may be said to convey the whole of the available relevant information supplied by the sample. Such solutions are termed efficient statistics. Other solutions, such as are exemplified by methods (a) and (b), convey only a determinate fraction of the available information; their efficiency is measured by the fraction which they convey, and this fraction may be measured by the ratio which the minimum random sampling variance bears to the actual variance appropriate to each statistic. Fig. 1 shows for all values of  $\sqrt{x}$  from 0 to 1 the actual efficiency of the methods of solution (a) and (b).

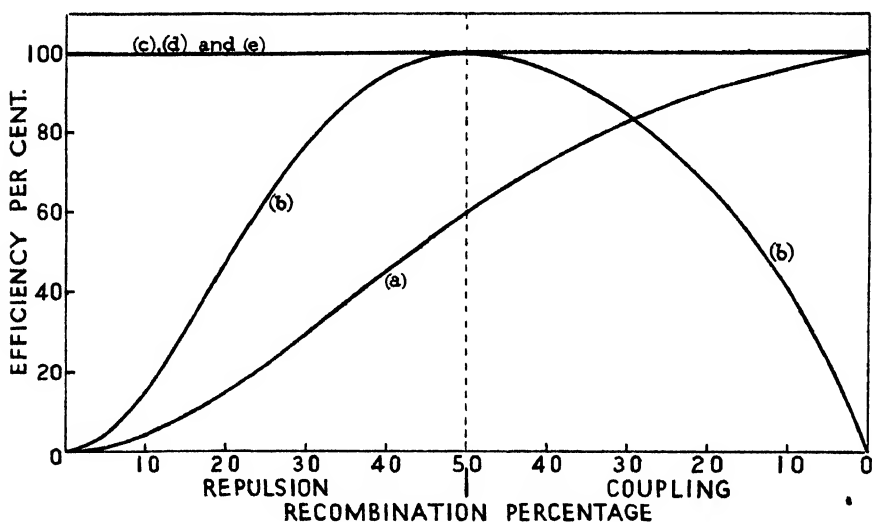


Fig. 1.

Since no statistic can exist of which the random sampling variance is less than the expression (3), this expression effectively specifies the amount of information, relevant to the value of  $x$ , which the data contain. Actually the quantity of information may be consistently measured, in large samples, by the inverse expression

$$I = \frac{n(1+2x)}{2x(1-x)(2+x)}.$$

By the aid of this quantitative measure of the amount of information, it will be seen that the efficiency of any statistic is simply the ratio of the quantity of information which it conveys, to the quantity of information latent in the data from which it was derived. To obtain the

corresponding quantitative measure of the amount of information relevant to the recombination fraction, the above expression should be multiplied by  $4x$ . The expression then shows the extent of the advantage of coupling over repulsion experiments for all recombination values; or, equally, the ratio of the numbers of seedlings to be counted to obtain equal precision.

It will be seen that each of the methods (*a*) and (*b*) attains 100 per cent. efficiency in special circumstances. Method (*a*) is perfectly efficient when  $x = 1$ , that is for close linkage measured by coupling. It is probable that this fact has led to its use in other circumstances, such as close linkage measured by repulsion, for which it is totally unfitted. For the case of our example its efficiency is less than 13 per cent., and its use for estimating linkage is equivalent to disregarding nearly seven-eighths of the data. Method (*b*) is perfectly efficient at  $\sqrt{x} = \cdot 5$ , or in the important group of cases in which crossing over is so frequent that linkage can scarcely be detected; it supplies a quick and useful test of the *significance* of apparent linkage, namely that the square of  $(a - 3b - 3c + 9d)$  should exceed  $36n$ , but is a bad method for the estimation of close linkage. Method (*c*), like methods (*d*) and (*e*), is perfectly efficient for all values of  $x$ .

The method (*d*) of maximum likelihood<sup>(3, 4)</sup> consists in maximising a quantity which can be written down by multiplying each observed number by the logarithm of the expectation. Thus

$a \log (2 + x) + b \log (1 - x) + c \log (1 - x) + d \log x$   
is a maximum, if

$$\frac{a}{2+x} + \frac{d}{x} = \frac{b+c}{1-x},$$

leading to the quadratic equation for  $x$

$$nx^2 - (a - 2b - 2c - d)x - 2d = 0,$$

or

$$3839x^2 + 1655x - 64 = 0,$$

of which the positive solution is

$$x = \cdot 035712,$$

the recombination percentage is 18.898, and the comparison of expected with observed frequencies is

	Starchy		Sugary		
	Green	White	Green	White	Total
Expected ( <i>m</i> )	1953.775	925.475	925.475	34.275	3839
Observed	1997	906	904	32	3839
Difference ( $\delta$ )	+43.225	-19.475	-21.475	-2.275	0
$\delta^2/m$	.9563	.4098	.4983	.1510	2.61

It will be seen from the fact that the expectations differ from those of method (c) by only about one-sixteenth of a seedling in each class, that this solution agrees closely with that already obtained. It has indeed been proved that, at least in the theory of large samples, all efficient solutions will be equivalent. We may therefore anticipate that a third scarcely distinguishable solution will be obtained by the procedure (e) of making  $\chi^2$  a minimum.

The measure of discrepancy  $\chi^2$  may be expressed as an explicit function of  $x$  in the form

$$\chi^2 = \frac{4}{n} \left( \frac{a^2}{2+x} + \frac{b^2}{1-x} + \frac{c^2}{1-x} + \frac{d^2}{x} \right) - n;$$

the condition that this should be a minimum leads to a quartic equation for  $x$ , of which the solution in our example is

$$x = \cdot035785,$$

giving a recombination value of 18.917.

The comparison of expected with observed frequencies is

	Starchy		Sugary		
	Green	White	Green	White	Total
Expected ( $m$ )	1953.845	925.405	925.405	34.345	3839
Observed	1997	906	904	32	3839
Difference ( $\delta$ )	+43.155	-19.405	-21.405	-2.345	0
$\delta^2/m$	.9532	.4069	.4951	.1601	2.0153

The results obtained by the five methods employed are summarised in the following table:

	Recombination value obtained	Standard error $x = \cdot0357$	$\chi^2$	Method
(a)	23.880	4.268	9.7170	Sum of complementary classes
(b)	21.260	2.348	3.8600	Weighted mean
(c)	18.880	1.545	2.0158	Product
(d)	18.898	1.545	2.0154	Maximum likelihood
(e)	18.917	1.545	2.0153	Minimum $\chi^2$

To make these comparable the standard errors have now all been calculated for the same value of  $x$ .

4. ADVANTAGES OF DIFFERENT METHODS OF ESTIMATION.

The principal advantages of the efficient group of statistics are:

(i) They provide better estimates of the linkage. The value 23.88 per cent. obtained by method (a) is not only a bad estimate from the data; it is an estimate which the data, when properly interpreted, firmly repudiate. Indeed it differs from the value indicated by the data



by more than three times the real standard error. The observations are in fact inconsistent with any real value much over 22 per cent., and to derive a value of over 23 per cent. from them is totally to misinterpret their evidence.

(ii) It has been shown<sup>(5)</sup> that  $\chi^2$  measures the discrepancy between observation and hypothesis only when efficient statistics are used. If the estimation is carried out by less efficient methods, then  $\chi^2$  will be affected by errors of fitting of the same order as the errors of random sampling, and will not provide any real test of the hypothesis. In the present example the values of  $\chi^2$  found by using efficient statistics show that there is no sign of a significant departure from expectation; while using method (a) the investigator is inevitably led to believe that very significant irregularities are present. This misleads him in two ways. On the one hand, he seems to have evidence of differential viability of the genotypes concerned, and on the other, whether he ascribes the apparent irregularities to differential viability or not, a cautious man will not place much reliance on estimates of linkage in which one or more of the frequencies have apparently been disturbed from their theoretical relationship. Observations therefore which are in reality free of differential viability, or other causes of error, may, by the mere inefficiency of the method of estimation, be discredited, or regarded as evidence that differential mortality occurs.

(iii) The efficient group of statistics agree so closely among themselves that no practical difference is to be expected between the conclusions drawn by using the different methods. This property follows from the theorem that the correlation between any two efficient statistics tends to +1.0 as the size of the sample is increased. With tolerably large samples it can therefore be expected that all efficient methods will be practically equivalent.

In ease of calculation, (c) and (d) which lead to quadratic equations are distinctly superior to (e) which leads to a biquadratic. Of the quadratics that of the maximum likelihood method is the simpler to write down, and should never take more than a few minutes to solve. Method (c) is, however, capable of simplification by tabulating the values of the product ratio for different recombination percentages. For this purpose we give such a table (p. 91) of these ratios and their logarithms. Thus in our example the ratio is .078025, and the table at once shows that the recombination per cent. is nearly 19. Direct interpolation gives 18.876. Alternatively, some will find it more convenient to use the logarithm 8.89223 for interpolation in the column of logarithms provided;

this gives 18.882; both values being amply near enough to the exact value 18.880. Logarithmic interpolation is much the more accurate for high linkage.

In the numerous genetic problems analogous to the one treated as a detailed example in this note, the method of maximum likelihood may be conveniently used to obtain at least one method of solution of known efficiency. If other methods are also efficient this will only be known by comparing the standard sampling error with that of the maximum likelihood solution. In general it may be said that such differences as exist between the different efficient statistics are always to the theoretical advantage of the maximum likelihood solution.

A good example of this may be illustrated in the present case by a previously unsuspected connection between the measure of discrepancy and the maximum likelihood solution.

It is now well known that although in the distribution of a given number of individuals among four classes, there are three degrees of freedom, yet if, as in the present problem, the expected numbers have been calculated from those observed by means of an adjustable parameter ( $x$ ), then only two degrees of freedom remain in which observation can differ from hypothesis<sup>(6)</sup>. Consequently the value of  $\chi^2$ , calculated in such a case, is to be compared with the values characteristic of its distribution for two degrees of freedom. This principle has been disputed, but the commonsense considerations upon which it was based have since received complete theoretical verification. In the present instance we can in fact identify the two degrees of freedom concerned. For the observed numbers in each class will be entirely specified if we know:

- (i) the number in the sample;
- (ii) the ratio of starchy to sugary plants;
- (iii) the ratio of green to white base leaf;
- (iv) the intensity of linkage.

Now if the expected series agrees in items (i) and (iv), it can only differ in items (ii) and (iii), and these will be completely specified by the two quantities  $p$  and  $q$  defined by

$$\begin{aligned} p &= a + b - 3c - 3d, \\ q &= a - 3b + c - 3d, \end{aligned}$$

specifying the ratios by linear functions of the frequencies.

The mean values of  $p$  and  $q$  will be zero, and the random sampling variance of each will be  $3n$ .

In the absence of linkage their deviations will be independent, but if linkage is present the mean value of  $pq$  may be found to be

$$-3n \frac{1-4x}{3},$$

or, the correlation between  $p$  and  $q$  is

$$\rho = -\frac{1-4x}{3}.$$

The simultaneous deviation of  $p$  and  $q$  from zero will therefore be measured by

$$\begin{aligned} Q^2 &= \frac{1}{3n} \left\{ \frac{1}{1-\rho^2} (p^2 - 2\rho pq + q^2) \right\} \\ &= \frac{3}{8(1-x)(1+2x)n} \left\{ p^2 + q^2 + \frac{2}{3}(1-4x)pq \right\}. \end{aligned}$$

This expression, which of course depends upon  $x$ , is a quadratic function of the frequencies; in this it resembles  $\chi^2$ , and on comparing term by term the two expressions it appears that

$$\chi^2 = Q^2 + \frac{1}{I} \left\{ \frac{a}{2+x} - \frac{b+c}{1-x} + \frac{d}{x} \right\}^2,$$

where  $I$  is the quantity of information contained in the data, as defined in Section 3.

This identity has two important consequences; first, that  $\chi^2 = Q^2$  for the particular value of  $x$  given by the equation of maximum likelihood, and for no other value. At this point then, even for finite samples, the deviations between observation and expectation represent precisely the deviations in the single factor ratios. The large value of  $\chi^2$  obtained by solution ( $\alpha$ ) could be seen at once to be fictitious from the fact that  $p$  and  $q$ , being +95 and +87 respectively, are not large compared to their standard error  $\sqrt{3n} = 107.3$ .

The second point is that for any value of  $x$ ,  $\chi^2$  is the sum of two positive parts of which one is  $Q^2$ , while the other measures the deviation of the value of  $x$  considered from the maximum likelihood solution; this latter part is the contribution to  $\chi^2$  of errors of estimation, while the agreement of observation with hypothesis is measured by  $Q^2$  only.

Fig. 2 shows the values of  $\chi^2$  and  $Q^2$  over the region covering the three efficient solutions.

The contact of the graphs at the maximum likelihood solution, makes it evident why the solution based on minimum  $\chi^2$  should be of no special interest, although  $\chi^2$  is a valid measure of discrepancy between observation

and hypothesis. As the hypothetical value,  $x$ , is changed, the value of  $Q^2$  changes, and, although this change is very minute, it gives the line a sufficient slope to make an appreciable shift in the point of contact. The solution of the minimum  $\chi^2$  equation will in fact always be slightly biased in whichever deviation tends to diminish  $Q^2$ , although the deviations to which  $Q^2$  is due have nothing to do with linkage.

Finally there is one advantageous property possessed by the product formula (c) which deserves the attention of geneticists. If one of the factors concerned, but not the other, has an appreciable effect upon viability, this circumstance will affect the two products equally, and

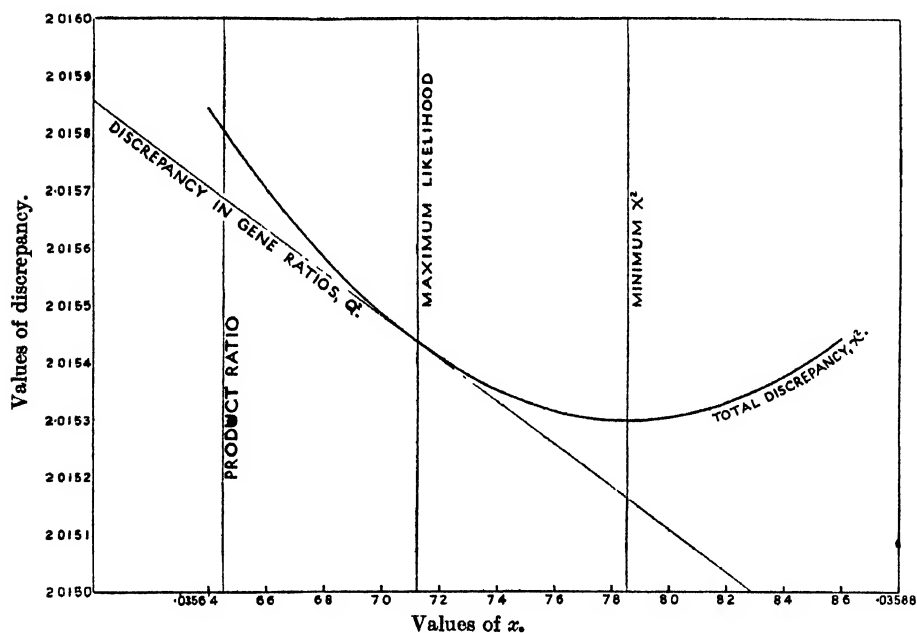


Fig. 2.

have no influence upon their ratio. Consequently the product formula is uninfluenced by such a source of error. Of course, it is also unaffected if both factors affect viability provided the percentage loss due to each factor is independent of the presence of the other, but we should require positive and independent evidence that this is so, in cases in which both factors affect the viability.

## 5. SUMMARY:

Five methods of solution are given of the statistical problem presented by typical linkage data. The example chosen shows the various errors

into which the use of inefficient statistics leads. Of the efficient methods the method of maximum likelihood possesses the advantage that it may be applied directly to any analogous problem, and is related in a previously unsuspected way to the measure of discrepancy  $\chi^2$ . The product ratio method, for using which a table is provided, enjoys the practical advantages of other efficient solutions, and is in addition unaffected by differential viability, if this is caused by one factor only. The method of minimum  $\chi^2$ , unlike the other two, is laborious in computation, and seems to possess no special theoretical interest.

*Table for use of product formula.*

Re-combination %	Coupling		Repulsion		Re-combination %	Coupling		Repulsion	
	Ratio of products <i>bc/ad</i>	Log + 10	Ratio of products <i>ad/bc</i>	Log + 10		Ratio of products <i>bc/ad</i>	Log + 10	Ratio of products <i>ad/bc</i>	Log + 10
1	·01356	6·13216	·020005	6·30114	26	·1467	9·16646	·16077	9·20621
2	·05516	6·74158	·080080	6·90352	27	·1616	9·20855	·17581	9·24504
3	·01262	7·10116	·018041	7·25626	28	·1777	9·24959	·19185	9·28296
4	·02283	7·35847	·032128	7·50688	29	·1948	9·28961	·20894	9·32002
5	·03629	7·55979	·050314	7·70169	30	·2132	9·32875	·22715	9·35631
6	·05318	7·72572	·072652	7·86125	31	·2328	9·36704	·24654	9·39189
7	·07366	7·86724	·090210	7·99656	32	·2538	9·40454	·26721	9·42685
8	·09793	7·99091	·013007	8·11418	33	·2763	9·44133	·28987	9·46123
9	·01262	8·10099	·016532	8·21833	34	·3002	9·47748	·31268	9·49510
10	·01586	8·20030	·020508	8·31192	35	·3258	9·51302	·33767	9·52849
11	·01954	8·29099	·024946	8·39700	36	·3532	9·54798	·36431	9·56147
12	·02375	8·37566	·029861	8·47510	37	·3823	9·58245	·39270	9·59406
13	·02832	8·45211	·035268	8·54738	38	·4135	9·61643	·42300	9·62634
14	·03347	8·52460	·041183	8·61472	39	·4467	9·64999	·45531	9·65831
15	·03915	8·59272	·047625	8·67784	40	·4821	9·68315	·48980	9·69002
16	·04540	8·65706	·054616	8·73732	41	·5199	9·71595	·52663	9·72151
17	·05240	8·71933	·062177	8·79363	42	·5603	9·74841	·56598	9·75280
18	·05972	8·77616	·070334	8·84717	43	·6034	9·78058	·60806	9·78395
19	·06787	8·83165	·079112	8·89824	44	·6494	9·81249	·65307	9·81496
20	·07670	8·88482	·088542	8·94715	45	·6985	9·84417	·70126	9·84588
21	·08628	8·93591	·098654	8·99411	46	·7510	9·87563	·75289	9·87673
22	·09663	8·98512	·109480	9·03933	47	·8071	9·90692	·80824	9·90754
23	·10780	9·03262	·121070	9·08304	48	·8671	9·93806	·86763	9·93833
24	·11984	9·07860	·133450	9·12532	49	·9313	9·96908	·93142	9·96915
25	·13279	9·12317	·146670	9·16634	50	1·0000	10·00000	1·00000	1·00000

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# THE VARIABILITY OF SPECIES IN THE LEPIDOPTERA, WITH REFERENCE TO ABUNDANCE AND SEX

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[Read June 6th, 1928.]

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### (1). INTRODUCTION. [E. B. F.]

THE present paper contains the results of recent work which the writers have carried out independently. They have, on the one hand, studied the variation to be expected theoretically in a population exhibiting Mendelian inheritance under the influence of selection, and, on the other, investigated the variability of certain Lepidoptera, chiefly common British moths. The conclusions at which they have arrived showed a somewhat striking agreement, and have already been published as a preliminary note (Fisher and Ford, 1926). It is now proposed to discuss them in greater detail and to publish the data.

Prof. E. B. Poulton, F.R.S., has given us much valuable help, and we have to thank him for placing the resources of the Hope Department, University of Oxford, at our disposal, also for authorising a grant in aid of these researches, from the Oxford University Fund for promoting the Study of Organic and Social Evolution. We are further indebted to him for writing the Appendix to this paper. One of us (E. B. F.) is indebted to the Department of Scientific and Industrial Research for a grant during the time that this and other work was in progress.

## (2). THE PROBLEMS INVESTIGATED. [E. B. F.]

One of the more important features of variation in the Lepidoptera is the remarkable unisexual polymorphism so frequent in that group. This has received most attention in the Rhopalocera owing to its connection with mimicry, but it also occurs among the Heterocera. Such polymorphism is by no means equally distributed between the sexes, for it is certainly more frequent in the female than in the male. Indeed we are unable to find a single instance of unisexual polymorphism of the male among mimetic butterflies, while in the female it is a frequent phenomenon in all parts of the tropics. Among the non-mimetic species the balance is again greatly in favour of the female, but not to the same extent; we shall return to this point in Section 6.

The importance of female mimicry has been emphasised by a number of workers; see, for example, the classical papers of Bates (1862), Wallace (1865), Trimen (1869), and Poulton (1916). Reference should be made to the latter paper for a discussion of the general bearings of the problem. Prof. Poulton suggests that it is an expression of a general tendency towards a greater female variability among butterflies as a whole, and points out that this conclusion is supported by certain differences in pattern which commonly exist between the sexes. In male butterflies the passage from one colour to another generally takes place suddenly, scales of one colour being replaced by those of another. In the case of females the change is gradual; an area in which the differently coloured scales are mixed in varying proportions occurs where two patches of colour join. In males the edges of the patterns thus tend to be hard (eulegmic) as compared with females, in which they are soft (dyslegmic). This would seem to indicate a generally greater instability of pattern in the latter sex, and is noticeable in the patterns of mimics as compared with models. Prof. Poulton says, "Dyslegmic patterns are not only common in mimics as compared with models, but are also common in females as compared with males. Thus the mimetic female forms of *Papilio dardanus* [Brown] bear in this respect the same relation to their Danaine models that they bear to their own male, and the female of the non-mimetic ancestor *P. meriones* [Feld.] in Madagascar is also far less eulegmic than its male."

The present work was originally designed to test by measurements of colour whether or not this marked excess of unisexual polymorphism in the female can be correlated with a relatively greater female variability in the group as a whole. In addition it was found that the data accumulated for this purpose supported certain theoretical conclusions on the relative variability of abundant and rare species; see Section 5.

## (3). METHODS AND MATERIAL. [E. B. F.]

For the above purpose it was considered advisable to estimate the variation of the character chiefly concerned in unisexual polymorphism, *i.e.* wing-colour, in forms not known to exhibit this phenomenon. In this way any differential variability of the sexes might be detected. It is further desirable to study non-mimetic species, as there is strong ground for supposing that female variability may have a special selective value in mimetic forms (see Section 6). Accordingly a number of British moths, chiefly belonging to the NOCTUIDÆ, were chosen as suitable material.

The method employed was to match the ground-colour of the fore-wings against a colour chart ranging, for example, from white to black, and divided into twelve stages, which were made as nearly as possible equivalent. This was done by adding, between each stage, a given quantity of black paint

(in drops) to a fixed amount of white paint, calculated so that the last stage is not distinguishable from pure black. The first stage, numbered 1, being left white and the last, 12, being black.

The writers are aware of the disadvantages and unavoidable inaccuracies of any direct method of matching colours (particularly when it is necessary to arrive at an average value for a varied field). These difficulties have been clearly stated by Sumner (1927), and some modification of the Ives Tint Photometer recommended by him might possibly be applicable in this case. It is hoped that some worker may be sufficiently interested to experiment in this direction in the future. At the same time it must be remembered that no method which might in any way damage the specimens is admissible in examining large quantities of museum material. We have, however, taken the greatest care to standardise the methods used in the present work, and we are satisfied that they are within the limits of accuracy required to demonstrate our results, as the arbitrary characteristics of the colour scale have largely been eliminated by the mathematical treatment (Section 4, (1)).

All matching was done as far as possible in a uniform light, and tests were made to estimate the accuracy attained. It was found that only rarely were individuals placed in different groups upon a second independent examination. The entire data giving the original grouping, together with the results of examining certain species a second time for tests of accuracy, have been deposited in the Natural History Museum, South Kensington, in case they should be of use to any worker in the future and to avoid the high cost of excessive tabulation in the present paper.

It was not possible to compare all ground-colours with the same chart. Thus a number of different charts, ranging between different colours, were employed. In this way 54 species, giving a total of 7605 specimens, were studied. It was, however, found impossible to compare in any satisfactory way the results of matching against different charts. For this reason only those species matched against a single chart are considered in the present paper. This is the one with which the largest number had been compared. It was made in the way described above, but in two parts. Stages 2-7 being made by regular combinations of white with the shade of dark brown marked Vandyke Brown in Ridgway's Colour Chart (Ridgway, 1912), 8-12 being similar combinations of this colour with black. Treated in this way it was found that this particular shade gave a graded series from white to black well within the error of matching, and bringing in the necessary brown tint. This restriction reduces the number of species to 35, and the number of specimens to 5227; these are tabulated in Sections 4 and 9.

Care was exercised in regard to the insects studied. It was, of course, only possible to use species which vary to a measurable extent, and which could be obtained in some numbers. In double (or more) brooded species only those from the same brood were utilised. Further, in all cases where local variation was seen to exist, determinations were made only from specimens caught in the same locality. These restrictions reduced the available material very considerably.

Collections of Lepidoptera often give an exaggerated impression of the variability of any species, owing to the collectors tending to keep the extremes more frequently than the typical forms. This tendency will be at its minimum when the intention is to obtain large samples of the species, and it is from such collections that the present material is principally drawn. It seems improbable that it will introduce any error in the comparison of the variability either between the sexes or between species of varying abundance.

## (4). STATISTICAL EXAMINATION OF THE DATA. [R. A. F.]

Taking the actual grades used in classification as empirical units of colour density, the mean and standard deviation of each sex were obtained by direct calculation (see Section 9). Shepherd's adjustment was not used, since this would not allow for the grading errors mentioned above. On comparing the sexes it is at once apparent that the female is generally both darker, and, so far as the standard deviation may be accepted, more variable than the male. Of the 35 species only one shows a male both lighter and less variable than the female, in both respects the difference being insignificant, so that a second sample might well reverse the difference. In three other cases the male, though less variable, is the darker, to an insignificant extent; in three more the male though lighter is more variable, and of these one, *Feltia clerica*, Butl., must be judged significantly more variable in the male. The remaining 28 species are both darker and more variable in the female.

In addition to the sex-comparison the species were classified in broad categories according to the two characteristics of Abundance and Range. Such a classification must necessarily be very imperfect. Thus 10 species could be placed in the most abundant group under the designations "abundant" or "very common," 12 more can be counted as "common," while the remaining 13 fall under such designations as "rather common," "common locally," "rather local," "local" and "rare." In respect of range the two most widespread fall into the class comprising "cosmopolitan," "holartic" and "old world" distributions; 18 may be classed as "palaeartic," though, of course, their range is often less than the whole of this region; 13 may be classed as "European" in the same broad sense, while the remaining two are confined to

TABLE I.  
CLASSIFICATION OF SPECIES BY ABUNDANCE AND RANGE.

	A. Abundant.	B. Common.	C. Less than common.
1. Cosmopolitan, etc.		<i>Hydroecia nictitans</i> , Bork.	<i>Agrotis saucia</i> , Hb.
2. Palaeartic	<i>Agrotis segetum</i> , Schiff. <i>Apamea secalis</i> , L. <i>Orrhodia vaccinii</i> , L. <i>Triphaena pronuba</i> , L. <i>Taeniocampa gothica</i> , L. <i>Taeniocampa incerta</i> , Hufn. <i>Xylophasia monoglypha</i> , Hufn.	<i>Agrotis corticea</i> , Hb. <i>Agrotis tritici</i> , L. <i>Bombycia viminalis</i> , Fb. <i>Dianthoecia carpophaga</i> , Bork. <i>Mamestra dentina</i> , Esp. <i>Mamestra pisi</i> , L. <i>Selenia bilunaria</i> , Esp.	<i>Agrotis puta</i> , Hb. <i>Eumichtis adusta</i> , Esp. <i>Miana bicoloria</i> , Vill. <i>Noctua dahlis</i> , Hb.
3. European	<i>Miana strigilis</i> , View. <i>Noctua xanthographa</i> , Fb. <i>Taeniocampa stabilis</i> , View.	<i>Amathes lychnidis</i> , Schiff. <i>Luperina testacea</i> , Hb. <i>Noctua rubi</i> , View.	<i>Agrotis agathina</i> , Dup. <i>Agrotis cinerea</i> , Hb. <i>Agrotis cursoria</i> , Bork. <i>Agrotis vestigialis</i> , Hufn. <i>Omphaloscelis lunosa</i> , Haw. <i>Selidosema ericetaria</i> , Vill. <i>Taeniocampa munda</i> , Esp.
4. Insular		<i>Feltia clerica</i> , Butl.	<i>Hydroecia crinanensis</i> , Burrows.

Great Britain and to the Falkland Islands respectively. The classification actually adopted is given in full in Table 1.

An examination of the average values of coloration in each class of the table, as set out below :—

TABLE 2.

Males.					Females.				
	A.	B.	C.	Mean.		A.	B.	C.	Mean.
1	—	5.42	6.89	6.16	1	—	6.15	7.28	6.72
2	5.93	4.52	4.80	5.13	2	7.35	5.66	6.98	6.61
3	5.83	6.12	4.86	5.50	3	7.04	6.76	6.27	6.09
4	—	7.42	5.33	6.38	4	—	9.48	5.66	7.57
Mean	5.90	5.24	5.03	5.35	Mean	7.26	6.29	6.51	6.65

shows that while there is no appreciable association between Range and pigmentation, Abundance is apparently so associated, for the abundant species are on the average distinctly darker than the remaining species. The averages of the standard deviations may be tabulated in the same way :—

TABLE 3.

Males.					Females.				
	A.	B.	C.	Mean		A.	B.	C.	Mean.
1	—	1.051	1.955	1.503	1	—	1.237	2.159	1.698
2	1.594	0.908	1.146	1.228	2	1.887	1.414	1.376	1.584
3	1.509	1.257	0.833	1.009	3	1.758	1.629	1.346	1.399
4	—	1.867	0.952	1.410	4	—	1.460	0.912	1.186
Mean	1.568	1.087	1.025	1.201	Mean	1.848	1.457	1.385	1.542

Setting aside results obtained from only a single species, it is clear that in both sexes the variability, as measured so far, is greater in the more abundant species, and also to some extent in those with larger ranges. In spite of the very rough and provisional character of both classifications it is evident that it has not been sufficiently imperfect wholly to obscure the association of variability with abundance. With range the case is different; the apparent association might be due wholly to chance in the choice of the species examined; on the other hand, any real association of variability with range will certainly have been much obscured by the broad classification which it is necessary to use.

#### (4. 1.) Elimination of the Arbitrary Characteristics of the Colour Scale. [R. A. F.]

The comparisons of variability made so far are open to a most serious objection, for the comparisons we wish to make, between male and female, or between more and less abundant species, have in fact to be made between groups of insects with a different average depth of pigmentation; in each case the group apparently the more variable is certainly the darker. Consequently, we must consider the possibility that the apparent differences in variability are in whole or in part fictitious, owing to the grades at the darker end of the scale not having the same intrinsic value as those at the lighter end. The

difficulty is a fundamental one, since we have no criterion as to what would constitute intrinsically equal grades. It may indeed be supposed that for each species there exists a natural pigmentation scale such that equal genetic changes produce on the average equal effects in different parts of the scale; and we have no reason to assume that this scale will not be substantially the same for the different species. However, we have no means of calibrating the scale of grades actually used with such a natural scale of pigmentation; consequently, the only numerical conclusions which can be drawn from the data with certain validity must be such as to be independent of the relative values of the successive grades of our colour scale.

This it is possible, in some measure, to do, by taking account of the association between depth of pigment and variability within groups of species. For this purpose the classification by range may be ignored. For each of the three abundance classes, the values of the mean and of the standard deviation are diminished by the average values for the class, giving the deviations from the average; the sums of the squares of these deviations are then calculated for the species means, and the sums of the products for the mean and S.D. of each species. Any general tendency, whether due to the arbitrary characteristics of our colour scale, or not, for the darker species to appear the more variable, will be expressed by the ratio of the sum of the products to the sum of the squares. This ratio supplies a means of making allowance, in the comparison of two groups, for any difference there may be in the mean pigmentation, and if after making this allowance the one group is still more variable than the other, it may be concluded that approximately the same result would have been obtained, whatever system of colour grades had actually been employed. The following table shows the actual values obtained in calculating this allowance.

TABLE 4.

	Males.		Females.	
	Sum of Squares.	Sum of Products.	Sum of Squares.	Sum of Products.
Abundant . . . .	13.013	+5.0939	15.009	+1.9626
Common . . . .	18.888	+4.5609	32.768	+3.1099
Less than common . .	16.213	-0.4063	11.576	-0.5377
Total . . . .	48.114	+9.2485	59.353	+4.5348
Ratio . . . .	+0.1922		+0.0764	

In both sexes the sum of the products is positive, showing that either owing to the colour scale employed, or as a general fact in the group concerned, the darker species do generally appear to be the more variable. The difference between the ratios obtained for females and for males may be ascribed at least for the major part to the darker average tint of the former; the scale being apparently most open at about grade 7, and necessarily allowing less variability to show itself as the mean tint approaches the extreme grades.

The above method indicates that for each grade of colour an allowance of 0.1922 should be made in the standard deviation of the males, and of .0764 in that of the females. These allowances probably suffice amply to obtain results which shall be practically independent of the colour scale; however, in view of the difference in the allowance which appears to be necessary for the two

sexes it has been thought worth while to calculate afresh an allowance, to be the same in both sexes, which depends not only on the colour grade but on its square. Allowing each of the six classes made by the three degrees of abundance and the two sexes, to determine its own mean, the regression was found in the form

$$+ \cdot 51490x - \cdot 032943x^2$$

where  $x$  is the grade number. For the mean values of the males and females, 5.35 and 6.65, this formula is equivalent to the simple regressions 0.1624 and 0.0768, agreeing thus very satisfactorily with the two values previously obtained.

The mean standard deviations, taking allowance for the mean tint of each species come out as follows :—

TABLE 5.

Males.					Females.				
	A.	B.	C.	Mean.		A	B.	C	Mean
1	—	1.131	1.875	1.503	1	—	1.220	2.060	1.640
2	1.635	1.189	1.368	1.402	2	1.824	1.548	1.324	1.606
3	1.585	1.256	1.052	1.222	3	1.741	1.559	1.341	1.484
4	—	1.764	1.047	1.406	4	—	1.443	0.956	1.200
Mean	1.620	1.249	1.212	1.341	Mean	1.799	1.516	1.361	1.568

as reduced by the quadratic formula to equivalent standard deviations for grade 6. It is a matter of indifference, for the comparisons, to which grade the standardisation is made.

There is evidently still a very substantially greater variability among the females, after allowing for the fact that this variability is liable to be measured in a different part of the scale. Of the 35 species only 8 (against 27) have an adjusted standard deviation larger in the male, and in only 4 of these (against 18) does the difference exceed 0.25.

In consequence of the high correlation which exists between the variabilities of the males and females of the same species, the 35 differences between the sexes afford a precise test of the significance of the effect of sex. For other comparisons it is necessary to compare different species, and the sampling errors involve not merely the differences in sexual differentiation, but the much larger differences in variability which exist between different species. Hence although the differences are absolutely greater, their statistical significance is much less pronounced. The best available basis of comparison will be afforded by the variability observed between species classed alike both as to abundance and as to range. The estimates of the variance between species of the same class obtained in this way are 0.3802 for the standard deviations of males, and 0.2147 for that of females. In view of these values the difference between classes of different range cannot be judged significant, although the general tendency, in both sexes, for the species with the wider range to be more variable in the district sampled, suggests the possibility that there is here a real effect which more extensive data might bring out. With respect to abundance the difference in the females between the variability in the abundant class and that of the other two classes together exceeds twice its standard error, and must therefore be regarded as significant, in the sense of not being ascribable to chance in the selection of the species. The additional fact that in both

sexes the three classes come out in order of abundance gives additional certainty to this conclusion. Since the precision of such a test must lose much by the roughness of the criteria by which the species are classified, we may conclude that an exact classification based upon actual numbers would have shown even larger differences in variability.

(5). THE THEORETICAL CONNECTION BETWEEN VARIABILITY AND ABUNDANCE. [R. A. F.]

Charles Darwin (*Origin of Species*, chap. ii), in studying the causes of variability, attempted a statistical investigation of material relating to a large number of different species of plants. He was, perhaps unfortunately, dissuaded from publishing his actual tabulations, but gained the concurrence of Hooker to the general conclusions that "wide ranging, much diffused, and common species vary most." Darwin was concerned to show that it was not merely that wide-ranging forms give rise to local varieties in reaction to different inorganic and organic environments, but also that, "in any limited country, the species which are most common, that is, abound most in individuals, and the species which are most widely diffused within their own country (and this is a different consideration from wide range, and to a certain extent from commonness), oftenest give rise to varieties sufficiently well marked to have been recorded in botanical works."

It has since been pointed out (Fisher, 1922) that, assuming Mendelian inheritance, the individual variation in a character showing continuous variation may be regarded as an equilibrium condition between the action of mutations, tending to increase variability, and of selections tending to diminish it. If in such an equilibrium the mutation rates, and also the selection rates, were the same for all species, then the variance would be proportional to the population of the species. We know of no reason for supposing that the mutation rates are different for species differing in abundance, but it is *a priori* probable that in abundant species individual survival is less fortuitous, and more selective, than in rare species; while it is easily demonstrable that in species in which a higher proportion of the total variance is ascribable to genetic causes, the effective selection will be more intense than in species in which the variance is to a larger extent ascribable to environmental variations. On these grounds we should expect the more abundant species to be, in fact, the more variable, though to an extent which can only be ascertained by observation.

Since many other circumstances besides abundance must influence the variability, the effect of this factor can only be expected to show itself as a statistical average of a considerable group of species. The data for 35 species presented with this paper thus seem to provide a decided confirmation of the conclusion arrived at on purely theoretical grounds; moreover, the fact that Darwin's observation with respect to "well-marked variations" should be equally applicable to continuous variation would be unintelligible if, as is sometimes assumed, continuous variation had some origin distinct from the familiar Mendelian mutations.

The theory does not provide an explanation of the difference in variability between the two sexes. It is true that in so far as the variability is due to sex-linked factors, there will be some tendency, in moths, for which the female is heterogametic, for the female to be the more variable, but this explanation is insufficient numerically to explain the large differences actually observed.

It has been shown (Fisher, 1922) that the effect of complete dominance, in any factor in which the proportion of recessive genes is represented by  $q$  will



be to retard the action of selection simply in the ratio  $q : 1$ . If, to make the simplest possible assumption, selection were to act with equal intensity on the two sexes, the corresponding retardation of the selection of a sex-linked factor would be

$$\frac{1}{3}(1 + 2q),$$

for of three sex chromosomes, two will be in the male, and subject to retardation. Now in the male, or in any case with an autosome, the variance contributed by any factor will be proportional to the product of the frequencies of the two phenotypes, namely to

$$q^2(1 - q^2),$$

and since with uniform selection, the frequency in any element  $dz$  is proportional to the differential element

$$= \frac{dq}{q(1 - q)}$$

the expression for the variance in the autosomal case will involve the integral

$$\int_0^1 \frac{1}{q} \cdot q^2(1 - q^2) \frac{dq}{q(1 - q)} = \int_0^1 (1 + q) dq = \frac{3}{2},$$

while for the male, with sex-linked factors, we shall have

$$\int_0^1 \frac{3}{1 + 2q} \cdot q(1 + q) dq = \frac{3}{2} \left( 1 - \frac{1}{4} \log 3 \right).$$

For the female, the product of the frequencies of the two phenotypes is simply  $q(1 - q)$ , giving the integral

$$\int_0^1 \frac{3dq}{1 + 2q} = \frac{3}{2} \log 3.$$

Now  $\log 3 = 1.0986$  so that the variance of the female is increased by 9.86 per cent. over the autosomal case, while that in the male is diminished by 27.47 per cent. for factors in the sex chromosome. If one mutation in 10 appeared in the sex chromosome, the variance of the two sexes would be in the ratio 10.0986 : 9.7253, differing by only about 3.7 per cent. The differences observed are 21 per cent., 39 per cent. and 23 per cent. in the three abundance classes, and are consequently considerably greater than could be explained by sex-linked factors. The only modification of the above assumptions which would make much difference would be to throw the whole weight of selection on to the male. This would tend greatly to increase the amount of sex-linked variance. Since, however, it should be possible to determine experimentally how much of the heritable variability of a species is sex-linked, it is useless to discuss hypothetical possibilities in advance of the necessary facts.

#### (6). DISCUSSION OF THE GREATER VARIABILITY OF THE FEMALE SEX, AND RELATED PROBLEMS, IN THE LEPIDOPTERA. [E. B. F.]

The foregoing data have shown that the female moth is distinctly the more variable, the difference being about 30 per cent. for all degrees of abundance. It is probable that at least two main factors are responsible for this difference of variability between the sexes.

In all cases so far examined the female has proved to be the heterogametic sex in the *Lepidoptera* (see Schrader, 1928, p. 132), and it is reasonable to

suppose that this is a constant feature of the group. It has been demonstrated in the last Section that this condition must have the effect of increasing the variability of the female, but to an extent which is insufficient to account for the observed facts. We must therefore look for an additional cause for the excess of female variability.

It appears to us that this is to be found more in the physiological than in the genetic differences between the sexes. Many genetic factors react with the sex hormones, producing the well-known sex-limited inheritance. Further, it appears that the sex hormone of the male in the Lepidoptera has the effect of inhibiting the action of an undue proportion of the genes. For this there appears to be at present no explanation; but the fact seems to be evident from the frequency of unisexual polymorphism in the female, and its rarity in the male (as mentioned in Section 2) in those cases where the differences are large enough to be appreciated readily. We suggest that the excess of female variability, above the amount to be accounted for by sex-linkage, is an expression of this process operating in the group as a whole, and not merely in the more striking examples which have previously attracted attention.

That sex-controlled inheritance is of the ordinary Mendelian type, the operation of certain factor-pairs being inhibited in one of the sexes, has been clearly demonstrated for a number of species (*e.g.* by Fryer, 1913, for the mimetic *Papilio polytes*, L.; and by Goldschmidt und Fischer, 1922, for the non-mimetic *Argynnis paphia*, L.). As has previously been pointed out, this type of inheritance is common in mimetic butterflies, but the female is always the polymorphic sex; it appears actually to be unknown for the polymorphism to be restricted to the male. Female unisexual polymorphism is also found in large numbers of non-mimetic species, and in this case the same type of dimorphism may be present in many allied forms. For example: in the LYCAENIDAE numerous species have blue males and polymorphic females, these being either black or blue, with all intermediate stages; the reverse condition does not occur. In *Argynnis* and allied genera several species have monomorphic brown males with dimorphic brown or greenish-black females. In a number of species belonging to the genus *Colias* a similar condition occurs, the female being either yellow (like the male) or white. This has been worked out in detail by Gerould (1911) for the American *Colias philodice*, Godt., and shown to give a simple Mendelian segregation.

The last example appears to be of some importance, since it supports a possible alternative explanation of sex-controlled inheritance, which has some bearing on the present problem. This was put forward by Goldschmidt (1923). He says that "if there was a pigmentation factor in the Y chromosome which reacted together with the autosomal factor in such a way that the coloration only appeared in the simultaneous presence of both factors, the result would be the same [as the ordinary physiological interpretation], *i.e.* the coloration would only appear in the females, since they alone possessed the Y chromosome." He further says: "Evidence for the accuracy of this conception could be obtained if occasionally a factorial exchange took place between the X and Y chromosomes. In such a case males of the particular form would be possible whose heredity could easily be deduced. In the *Colias* form mentioned above white males are as a matter of fact occasionally observed (Gerould)." More recently a similar case has come to light in an exceptional white male of *Colias euzanthe*, Feld. (Hemming, 1926). There is thus some ground for Prof. Goldschmidt's hypothesis, although the hereditary behaviour of these exceptions is unknown. Considering, however, the unlikelihood of large numbers of autosomal genes having complementary factors in the Y chromosome, and that

rather rare examples of male unisexual polymorphism exist among non-mimetic species, as in the moth *Parasemia plantaginis*, L., the mechanism of variation which he suggests must be somewhat unusual.

We consider it possible, therefore, that sex-controlled inheritance is the result of two distinct processes. The one being due to one of the sex hormones inhibiting the action of certain genetic factors, the other (and almost certainly the rarer) to the interaction of genes in the Y chromosome with others in the autosomes. The latter type should give purely female unisexual polymorphism (except for occasional crossing-over); but it is extremely doubtful if it is sufficiently common to account for any considerable proportion of the observed excess of female variation. Why the action of the former type should result in more female than male unisexual polymorphism, as it appears to do, remains unexplained.

It must be mentioned that mimicry, and increased polymorphism of the female, of the type described above, is not confined to butterflies; it also occurs among day-flying moths. For example, PTEROTHYSANIDAE of the genus *Hibrides* have non-mimetic slightly dimorphic males; the females, which are totally different, being polymorphic and regarded as mimetic (*Proc. Ent. Soc. Lond.*, 1918, p. cxxxiv); see the Appendix to this paper.

In conclusion we may discuss the significance of unisexual polymorphism and allied phenomena from another point of view. It must first be noticed that many butterflies with monomorphic females are mimetic in this sex only, and depart widely from the typical coloration of the genus, which is retained by the non-mimetic males. Such species are, therefore, further examples of the greater female variability discussed previously.

Neither this condition, nor that of the true unisexual polymorphism found so commonly in mimetic species, is *a priori* to be expected from the mechanism described above, which controls the relatively greater female variation. We have shown that the female is the more variable sex (1) owing to the fact that it is heterogametic in the *Lepidoptera*, and (2) that a number of genetic factors interact with the male hormones to inhibit their appearance in that sex, and (3) possibly owing to the occasional presence of factors in the Y chromosome. But none of these conditions might be expected to produce a complete inhibition of mimetic pattern in the male, and its full development in the female. Their result should be to reduce the variability of the male, with the consequence that this sex should follow the general trend of variation in the female, but lag behind it; *i.e.* the males would be expected to mimic, but less perfectly than the females. This condition is frequently realised. Although often restricted to the females, mimicry is quite common in both sexes, when the female is frequently the better mimic, thus confirming the expectation.

It would thus appear that, added to the greater female variability described above, some other factor operates to inhibit the appearance of any mimicry in the males of many species. This we believe may be some form of sexual selection. One of the chief functions of the male is to stimulate the female to copulation; this is most frequently performed by sight or smell, sometimes by touch and hearing; but the displays of an insect often combine several of these types; Richards (1927) gives a valuable summary of this subject. It is probable, therefore, that in certain cases selection in favour of keeping the males to a relatively constant type, in order that their sex should be recognised by the female and cause her the appropriate stimulation, outstrips any advantage which they might derive from mimicry. That the male can often dispense with the latter advantage, in cases where it appears to be of value to the female, is possible for three reasons. (1) One of the most dangerous periods in the

life of the female, when she is most exposed and most in need of any protection which mimicry might afford, is during the time she is laying eggs; the male is probably never exposed to any danger of corresponding severity in the imago state. (2) Many females are less active on the wing than the males, presumably owing to the large size of the body. (3) Since a single male can fertilise a number of females, a proportion of the males could always be killed off without affecting the numbers in the next generation. See Wallace (1865) and Poulton (1909).

We therefore believe that selection of this kind may partly be responsible for the complete lack of development of a mimetic pattern in the males of so many mimetic species. Whether or not it can be responsible, to a small extent, for the greater female variability as a whole remains an open question; though this seems unlikely in the nocturnal species.

(7).

## SUMMARY.

1. The frequency distribution of depth of pigment in the ground-colour of the fore-wings of 35 species of British moths has been obtained by comparison of over 5000 specimens with a standard colour scale.

2. For comparison of variabilities of groups of different average tint the standard deviations have been adjusted to eliminate any arbitrary elements which might have been introduced by the scale employed.

3. The mean tint is darker in the females than in the males, and is also darker in the more abundant than in the less abundant species.

4. Even after adjustment the mean variance is about 30 per cent. higher in females than in males, and is in both sexes greatest in the abundant species, and least in those which are less than common.

5. It is also possible, though the difference is not in this material statistically significant, that the species with wider range are, in any one locality, the more variable.

6. The association of variability with abundance accords with an early generalisation of Darwin's, and with the theory that variability is determined by a balance between the influences of mutations and selection. This theory is insufficient numerically to account for the large differences in variability between the sexes.

7. In view of the frequency of polymorphism, and other marked variations, in the females as opposed to the males in Lepidoptera, it is suggested that the male sex hormones may inhibit the action of a number of the factors influencing the development of pigment, as in the well-known sex-controlled variation. The suggestion of Goldschmidt that there exist pigmentation factors in the Y chromosome capable of interaction with autosomal factors to cause pigmentary differentiation is an alternative view which may account for a few cases. This should result in purely female unisexual polymorphism [except for the possibility of occasional crossing-over between the X and Y chromosomes], but it is almost certainly an infrequent phenomenon. It is possible that sexual selection may, in part, be responsible for the complete inhibition of mimetic patterns in the males of certain mimetic species.

(8).

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(9). A LIST OF THE LEPIDOPTERA EXAMINED, GIVING THE MEAN AND STANDARD DEVIATION OF EACH SEX FOR THE COLOUR GRADES USED IN THE CLASSIFICATION. [R. A. F.]

	No. examined, ♂.	Males.		No. examined, ♀.	Females.	
		Mean.	S.D.		Mean.	S.D.
<i>Agrotis segetum</i> , Schiff. . . .	67	4.90	1.017	85	7.99	1.905
<i>Apamea secalis</i> , L. . . . .	120	6.82	2.526	105	7.75	2.615
<i>Orrhodia vaccinii</i> , L. . . . .	83	6.52	1.533	71	6.96	1.808
<i>Triphaena pronuba</i> , L. . . . .	127	6.17	1.463	147	6.63	1.672
<i>Taeniocampa gothica</i> , L. . . . .	146	4.40	1.105	157	9.05	1.458
<i>Taenicampa incerta</i> , Hufn. . . .	70	7.37	1.704	60	7.72	2.017
<i>Xylophasia monoglypha</i> , Hufn. . .	88	5.36	1.808	76	5.34	1.732
<i>Miana strigilis</i> , Clerck. . . . .	{ 80 137	7.145	2.480	{ 89 134	7.83	2.553
<i>Noctua xanthographa</i> , Fb. . . . .	105	6.48	1.415	62	8.32	1.607
<i>Taeniocampa stabilis</i> , View. . . .	92	3.87	0.632	117	4.98	1.114
<i>Hydroecia nictitans</i> , Bork. . . . .	71	5.42	1.051	48	6.15	1.237
<i>Agrotis corticea</i> , Hb. . . . .	111	4.78	1.344	75	7.44	1.742
<i>Agrotis tritici</i> , L. . . . .	89	5.63	0.993	79	7.56	2.080
<i>Bombycia viminalis</i> , Fb. . . . .	49	2.71	0.456	56	3.60	1.073
<i>Dianthoecia carpophaga</i> , Bork. . .	50	3.42	0.883	59	3.29	1.260
<i>Mamestra dentina</i> , Esp. . . . .	52	4.69	0.961	81	6.69	1.221
<i>Mamestra pist</i> , L. . . . .	54	5.56	0.984	52	6.31	1.229
<i>Selenia bilunaria</i> , Esp. . . . .	75	4.88	0.735	58	4.72	1.295
<i>Anathes lychnidis</i> , Schiff. . . . .	84	6.99	1.517	94	6.94	1.795
<i>Luperina testacea</i> , Hb. . . . .	76	5.80	1.592	67	6.49	1.870
<i>Noctua rubi</i> , View. . . . .	53	5.58	0.663	61	6.85	1.223
<i>Feltia clerica</i> , Butl. . . . .	64	7.42	1.867	33	9.48	1.460
<i>Agrotis saucia</i> , Hb. . . . .	80	6.89	1.955	58	7.28	2.159
<i>Agrotis puta</i> , Hb. . . . .	64	3.44	0.614	48	8.21	0.921
<i>Eumichlis adusta</i> , Esp. . . . .	41	6.10	0.663	47	7.13	1.153
<i>Miana bicoloria</i> , Vill. . . . .	64	4.53	2.631	65	5.43	2.318
<i>Noctua dahlia</i> , Hb. . . . .	35	5.11	0.676	30	7.14	1.113
<i>Agrotis agathina</i> , Dup. . . . .	59	5.46	0.502	49	6.10	0.941
<i>Agrotis cinerea</i> , Hb. . . . .	78	3.44	0.783	8	7.12	1.552
<i>Agrotis cursoria</i> , Bork. . . . .	46	4.89	0.900	54	5.50	1.270
<i>Agrotis vestigialis</i> , Hufn. . . . .	105	3.63	0.943	39	5.64	1.598
<i>Omphalocelis lunosa</i> , Haw. . . .	86	5.00	2.212	73	7.14	2.238
<i>Selidosema ericetaria</i> , Vill. . . .	56	7.00	0	24	7.25	0.676
<i>Taeniocampa munda</i> , Esp. . . . .	32	4.63	0.492	26	5.04	1.148
<i>Hydroecia crinanensis</i> , Burrows. .	54	5.33	0.952	97	5.66	0.912

(10).

## APPENDIX.

On the Male and Female Forms of the African Pterothysanid Moth *Hibrildes norax*, Druce. By Prof. E. B. POULTON. Plate XII.

When I read the typescript of my friends' paper it occurred to me that an account of the most polymorphic moth hitherto recognised would be of interest as an extreme example of variation in relation to sex. Of all known Heterocera, *Hibrildes norax* exhibits the nearest approach to such butterflies as *Papilio dardanus*, Brown, or *Charaxes etheocles*, Cram., with their numerous female forms. The females of the moth also follow those of the butterflies in their mimetic patterns, but any discussion of the degree of resemblance is better deferred until the forms themselves have been considered.

All the known forms of *H. norax* are represented on Pl. XII, which I owe to the great kindness and skill of my friend Dr. Eltringham. In this plate it will be seen that the differences between the males (figs. 1, 3, 5) are very small as compared with the females (figs. 2 (also 10), 4, 6, 7, 8, and 9). Nevertheless the males shown in figs. 1 and 5 were described as different species, *norax*, Druce, and *venosa*, Kirb., respectively, fig. 3 representing an intermediate form. Similarly figs. 2 (also 10), 6, 8, and 9 were respectively described as the species *crawshayi*, Butl., *ansorgei*, Kirb., *albescens*, Joic. & Talb., and *neavi*, Hampson. In addition to these, two new female forms *carpenteri* (fig. 4) and *sheffieldi* (fig. 7) are described in this appendix.

Sir George Hampson, in 1910, was the first to suggest, in the following passage, that these supposed species, so far as they were known at that date, were males and females of the single species *H. norax*.

"It seems possible that *H. venosa* is a variety of *norax* rather yellower with stronger streaks on the veins and an oblique shade across the apical area of fore-wing, and that *crawshayi*, *ansorgei*, and *neavi* are all forms of the female, the two former having the terminal band of hind-wing narrow; *crawshayi* has the fore-wing fulvous, the hind-wing with fulvous subterminal spots, *ansorgei* fuscous, with a broad whitish postmedian band, the hind-wing with white subterminal spots, whilst *neavi* has a very broad terminal band to hind-wing, the subterminal spots very small and either white or fulvous. All the forms were taken in one district by Mr. Neave except typical *ansorgei*, which he did not get, though some of the varieties approach it. . . ." (*P.Z.S.*, 1910, pp. 453, 454.) Dr. S. A. Neave, who had collected the moths which were the subject of Hampson's paper, wrote in confirmation:—"There is nothing in my experience in the field which would make improbable Sir George Hampson's suggestion that this species [*H. crawshayi*] and the new *H. neavi* are the females of *H. norax* and *venosa*" (*ibid.*, p. 454).

The evidence, brought forward above, that both the male forms and some of the female forms fly in the same area, has been much strengthened by the results obtained since 1910. The most striking of these are shown in the following table, which also includes, in the fourth locality (Petauke), a part of Dr. Neave's earliest material described in the 1910 *P.Z.S.*

It is reasonable to suppose that selection based on an increasing mimetic resemblance has played an essential part in developing and maintaining the three female forms *crawshayi*, *neavi* and *ansorgei*. Thus Dr. Neave writes of the first two:—

"Both *crawshayi* and *neavi* are remarkable Acraeine mimics, the former of *Acraea natalica*, Boisdu., the latter, with its heavy black margins to the secondaries, of *Acraea anemosa*, Hew.

Data of place and time. The specimens from the first and fourth localities are in the Hope Coll., the others in Brit. Mus.	♂ form <i>norax</i> (fig. 1).	♂ form <i>venosa</i> (fig. 5).	♀ form <i>crawshayi</i> (figs. 2, 10).	♀ form <i>neavi</i> * (fig. 9).	♀ form <i>ansorgei</i> (fig. 6).	Other ♀ forms.
Tang. Terr., Lulaguru (3766 ft.), 17 m. W. of Tabora. 1-31.xii.1917. G. D. H. Carpenter.	3	3	3		1	1 <i>carpenteri</i> , f.n. (fig. 4): like <i>ansorgei</i> , but with rich orange-ochreous F.W. bar.
N. W. Rhodesia, Solwezi. 1916 and 1917. H. C. Dollman.		1	1	1	1	
N.W. Rhodesia, Mwangwa. 1913 and 1914. H. C. Dollman.	1	2	1		2	
N.E. Rhodesia, E. Luangwa Distr., Petauke (2400 ft.). 1904 and 1905. S. A. Neave.	7	9 +1 in B.M.	6 +1 in B.M.	2		1 <i>sheffieldi</i> , f.n. (fig. 7): like <i>crawshayi</i> , but with much dusky suffusion of F.W. and dusky H.W.
Nyasaland, Mt. Mlanje. 1912-1914. S. A. Neave.		26	16		4	1 <i>albescens</i> , Joie. & Talb. (fig. 8): like <i>ansorgei</i> , but with white H.W.
Nyasaland, Mt. Mlanje, Luchunya R. 1913 and 1914. S. A. Neave.		9	7			
Port. E. Africa, Kola Valley. 5-8.iv.1913. S. A. Neave.		5	11		4	
Port. E. Africa, Ruu Valley. 2000 ft. 1913. S. A. Neave.		4	3			

\* A coloured figure of the ♀ f. *neavi* is given in *P.Z.S.*, 1910, pl. xxxviii, fig. 18.

"I found both these species of *Acraea* extremely abundant at Petauke in the Luangwa valley, whence most of the specimens of *crawshayi* and *neavi* come. These moths only resemble the large *Acraeas* fairly well on the wing as their flight differs a good deal. At rest, however, the resemblance is remarkable, the moth resting hanging from a grass stem, etc., with wings folded above its back, *exactly* as the *Acraea* does." (*P.Z.S.*, 1910, p. 454.)

The fact that *crawshayi* is far more abundant than *neavi*, and indeed than any other female form of *norax*, will be made evident by a glance at the above table. Its abundance is correlated with that of *Acraea natalica* among the large *Acraeas* of S. and E. Africa. Thus Dr. Neave writes of it in N. Rhodesia as "a common species everywhere and at all seasons, more especially in the Luangwa valley, where it is quite the most dominant of all the larger

Acraeinae" (*P.Z.S.*, 1910, p. 26). The difference between the patterns of these two female forms of *norax* is, however, small, as shown by comparing figs. 2 and 10 (*crawshayi*) with 9 (*neavi*). The Acraeinae models also exhibit a general resemblance to each other, a resemblance which may be so far intensified that the insects would probably be indistinguishable on the wing, as may be inferred from the representation, in *Trans. Ent. Soc. Lond.*, 1902, pl. xvi, of two butterflies taken by Dr. G. A. K. Marshall, F.R.S., in Mashonaland, fig. 10 being a male *anemosa*, and fig. 11 a female *natalica*. Dr. Marshall states that "the two species are very similar upon the wing, and that the resemblance is much closer in the case of the female *natalica* than the male, thus following the rule in mimicry, and confirming . . . the opinion . . . that the approach has been from the side of *natalica*." This latter species appears to have "adjusted markings of a type usual among Ethiopian *Acraeinae* in such a manner as to produce superficial similarity to *anemosa*, an *Acraea* in which a very remarkable and unusual appearance is the warning sign of exceptional defence against insect-eating animals" (*ibid.*, p. 493). Dr. Marshall indeed considers that *anemosa* is one of "the two best-protected members\* of the genus," the other being *A. zetes acara*, Hew. (p. 413). It is of much interest, therefore, that the ♀-f. *crawshayi*, mimicking the Acraeinae mimic, should be so much commoner than the ♀-f. *neavi*, mimicking the model—facts which may be reasonably explained by the far greater abundance of *natalica*.

It has already been pointed out on p. 381 that the mimicry of *Acraeas* by these female forms is especially developed on the under-surface and in the position of rest. The upper surface of the ♀-f. *neavi* (fig. 9), with its deeper sienna colouring and broad black margin to the hind-wing is, however, quite a good mimic of *anemosa*, while the paler sienna upper surface of *crawshayi* (figs. 2 and 10) may gain some advantage by a very superficial likeness to the *dorippus*, Kl., f. of *Danaida chrysippus*, L., and its mimics, such as the *inaria*, Cr., ♀ of *Hypolimnna misippus*, L.

This last suggestion receives some support from the fact that the only other ♀-f. which appears to be at all common, viz. *ansorgei*, was noted by Dr. Carpenter to be "on wing incipient *Aletis* or *chrysippus* mimic" (*Proc. Ent. Soc. Lond.*, 1918, p. cxxxiv). The specimen bearing this note is represented in fig. 6.

The three remaining female forms of *norax* are apparently extremely rare. I know of only two examples of *albescens*, one of *carpenteri* and one of *sheffieldi*.

The ♀-f. *albescens* (fig. 8) is a variety of *ansorgei* in which the pale sienna of the hind-wings has been replaced by white. On the wing it would probably present a superficial likeness to a black and white species of the Danaine genus *Amauris*, perhaps to a *Neptis* or to a day-flying moth (*Deilemera*).

The ♀-f. *carpenteri* (fig. 4) also represents a variety of *ansorgei*, described on p. 383. It does not seem to be a mimetic form.

The ♀-f. *sheffieldi* (fig. 7) is described on p. 383. The single example bears an MS. label, written by the captor, Dr. S. A. Neave, suggesting a possible mimetic resemblance to one of the female forms of *Acraea serena*, F. (*terpsichore*, L.)—an opinion supported by the comparatively small size of this female *Hibrides*.

There is a curious rough resemblance between the variational steps which appear to have led to certain female forms of *H. norax* and *Papilio dardanus*,

\* It is also probable that the greater variability of *natalica* has facilitated the mimetic approach from its side rather than from that of *anemosa*.



Brown, respectively—*ansorgei* representing *trophonius*, Westw.; *carpenteri*—*niobe*, Auriv.; and *albescens*—*hippocoon*, F. It is not to be supposed, however, that the sequences were the same; for, while there can be no doubt that *trophonius* arose from *trimeni*, Poult., the ancestral form of *hippocoon*, and itself gave rise to *niobe*, there are no grounds for the belief that the corresponding forms of the moth developed in a similar order.

*New female forms with a note on the ♀-f. albescens.*

*Hibrildes norax*, Druce, *carpenteri*, ♀-f.n.—This female form resembles *ansorgei*, but the broad postmedian band of F.W. is sienna instead of whitish. The tint of this band is similar to that of the hind-wing, which is somewhat deeper than in *ansorgei* and *crawshayi*, although paler than in *neavi*. The terminal band of H.W. is broader than in *ansorgei* and the subterminal spots smaller and sienna instead of white.

TYPE (Pl. XII, fig. 4) in Hope Dept., Oxford Univ. Museum. The single example was taken 10 December, 1917, by Dr. Carpenter at Lulanguru, Tang. Terr.

*Hibrildes norax*, *sheffieldi*, ♀-f.n.—This female form is described by Sir George Hampson as a variety of *crawshayi* in which "the fore-wing is suffused with fuscous leaving some ochreous white in cell, as streaks on inner area, and as a broad postmedian band, the whole hind-wing being suffused with fuscous" (*ibid.*, p. 453). The subterminal spots of the hind-wing are very pale ochreous.

TYPE (Pl. XII, fig. 7) in Hope Dept., Oxford Univ. Museum. The single example was taken 2 March, 1905, at Petauke, E. Luangwa District, N.E. Rhodesia, by Dr. Sheffield A. Neave.

The ♀-f. *albescens*, Joic. and Talb. (fig. 8), was described from a single example taken by Mr. T. A. Barns, April 1922 (end of wet season), on the E. bank of the Luvua River (alt. 3000 ft.), 85 m. N. of Lake Mweru. It is represented much reduced on pl. lxxx [Fig. 80], fig. 10, facing p. 264 of Barns' *Across the Great Craterland to the Congo*, London, 1923. A single male, only differing from typical *venosa* "in the broader apical band on the fore-wing," was taken by Mr. Barns, March 1922 (mid-wet season), in the East Luvua Valley (4000–5000 ft.), 5 days N.E. of Lake Mweru. The authors describe these insects as male and female of *Hibrildes ansorgei*, Kirby [*norax*, Druce], *albescens*, subsp. nov. (*Bull. Hill Mus.*, vol. i, No. 3, 1924, pp. 558, 559). Dr. Neave's example of the same form, here represented in fig. 8, and shown in the tabular statement on p. 381 to exist in the locality (Mt. Mlanje), where numbers of both male forms and four *ansorgei* females were taken, leaves no doubt about the validity of their conclusion, which the comparison of figs. 6 and 8 will render evident, the patterns being similar and the only difference a substitution of white for orange in the hind-wing. The proximity of Mr. Barns' locality to that in which Dr. Neave collected in 1904–5, and the occurrence of the ♀-f. *albescens* far to the east in Nyasaland, renders it improbable, however, that a separate geographical race of *norax* exists in the Luvua River district.

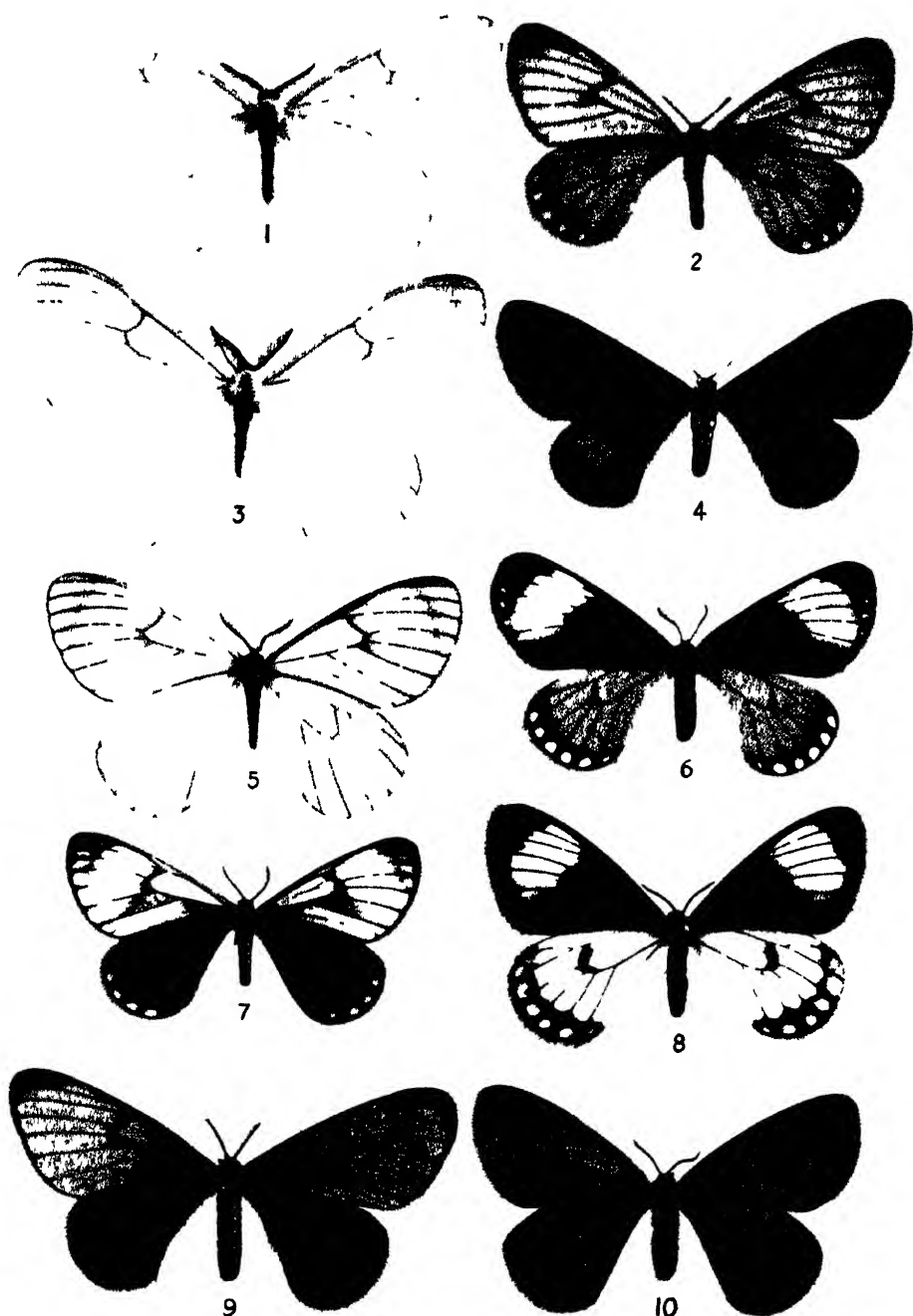
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## EXPLANATION OF PLATE XII.

All the figures are of the natural size.

Male and Female forms of *Hibrildes norax*, Druce (PTEROTHEYSANIDAE).

- FIG. 1. Male form *norax*, Druce: taken 20 December, 1917, at Lulanguru, 17 m. W. of Tabora, Tang. Terr., by G. D. H. Carpenter.
2. Female form *crawshayi*, Butl.: taken 6 December, 1917. Locality and captor as 1.
3. Male form *venosa*, Kirb., transitional to *norax*: taken 5 December, 1917, by the same captor in the same locality as 1.
4. Female form *carpenteri*, f.n.: taken 10 December, 1917. Locality and captor as 1. (The type.)
5. Male form *venosa*, Kirb.: taken 27 December, 1917. Locality and captor as 1.
6. Female form *ansorgei*, Kirb.: taken 4 December, 1917. Locality and captor as 1.
7. Female form *sheffieldi*, f.n.: taken 2 March, 1905, at Petauke (2400 ft.), E. Luangwa District, N.E. Rhodesia, by S. A. Neave. (The type.)
8. Female form *albescens*, Joic. & Talb.: taken 8 February, 1913, on Mt. Mlanje, Nyasaland, by S. A. Neave (B.M. register, 1914-171).
9. Female form *neavi*, Hmps. (Paratype): taken 1 February, 1905. Locality and captor as 7.
10. Female form *crawshayi*, Butl.: taken 14 February, 1905. Locality and captor as 7 and 9.



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THE AFRICAN PTERTHYSANID MOTH *HIBRIDIA VORAX* DRUCE

All the figures are of the natural size. Male (1, 3 & 5) and female (2, 4, 6, 8, 9 & 10) forms of *Hibridia vorax*. 1-6 are examples of forms taken in one locality; 7, 9 and 10 in another.



# THE APPLICATION OF THE "METHOD OF MAXIMUM LIKELIHOOD" TO THE ESTIMATION OF LINKAGE

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## INTRODUCTION

The "Method of Maximum Likelihood" is given by FISHER (1928) and is illustrated by a simple case of linkage between two factors. The method consists of multiplying each class frequency by the natural logarithm of its corresponding probability, summing, and determining the value for which the sum is a maximum. The linkage is expressed in terms of  $p$  where  $p$  is the proportion of gametes carrying both dominants plus the proportion of gametes carrying both recessives. The method is quite general, and may be applied to any type of ratio from which it is desired to estimate a linkage.

### A CASE INVOLVING COMPLEMENTARY FACTORS

BRUNSON (1) has studied maize families in which a factor for pale green seedling is linked with one of two complementary factors for aleurone colour, and he gives a formula, derived from EMERSON's formula, for the estimation of the linkage.

The  $F_2$  is classified as under, where  $C$  and  $R$  are complementary factors for aleurone colour, and  $P_{\sigma_1}$  is a factor for pale green seedling:

TABLE 1

*Frequencies observed in an  $F_2$  segregating for aleurone colour and pale green seedling (BRUNSON's data).*

	CR	Cr+cR+cr	SEEDLING TOTAL
$P_{\sigma_1}$	1907	1053	2960
$p_{\sigma_1}$	300	686	986
Aleurone total	2207	1739	n=3946

Then, considering only the factor  $P_{a_1}$  and the aleurone factor linked with it, the probabilities of the four classes in  $F_2$  will be:

$$\begin{array}{cccc} P_{a_1}A & p_{a_1}A & P_{a_1}a & p_{a_1}a \\ \frac{1}{4}(2+pp^1) & : \frac{1}{4}(1-pp^1) & : \frac{1}{4}(1-pp^1) & : \frac{1}{4}pp^1 \end{array}$$

where  $A$  is either  $C$  or  $R$ , and  $p$  is the proportion of  $(P_{a_1}A + p_{a_1}a)$  gametes in male gametogenesis, and  $p^1$  is the proportion of  $(P_{a_1}A + p_{a_1}a)$  gametes in female gametogenesis. The effect of the linkage is then entirely expressed in the term  $pp^1$ , and if crossing over is equal in male and female, the cross-over percentage will be  $(1 - \sqrt{pp^1}) \times 100$  in the coupling phase, and  $(\sqrt{pp^1}) \times 100$  in the repulsion phase. For simplicity,  $\theta$  will be written for  $pp^1$ , and since the data provide no evidence on the matter, it will be assumed that crossing over is equal in male and female. (See FISHER (1928)).

Then, bringing in the complementary factor for aleurone colour, we get the probabilities in the four classes:

$$\begin{array}{cccc} CRP_{a_1} & CRp_{a_1} & [Cr+cR+cr]P_{a_1} & [Cr+cR+cr]p_{a_1} \\ \frac{3}{16}(2+\theta) & \frac{3}{16}(1-\theta) & \frac{3}{16}(2-\theta) & \frac{1}{16}(1+3\theta) \end{array}$$

Then the logarithm of the likelihood will be:

$$\begin{aligned} L = 1907 \log \frac{3}{16}(2+\theta) + 300 \log \frac{3}{16}(1-\theta) + 1053 \log \frac{3}{16}(2-\theta) \\ + 686 \log \frac{1}{16}(1+3\theta). \end{aligned} \quad (1)$$

And the maximum likelihood value of  $\theta$  will be that for which the first differential of equation 1, with respect to  $\theta$ , is zero:

$$= \frac{1907}{2+\theta} - \frac{300}{1-\theta} - \frac{1053}{2-\theta} + \frac{2058}{1+3\theta} = 0. \quad (2)$$

Which becomes on multiplying out:

$$11838\theta^3 - 12802\theta^2 - 11376\theta + 8740 = 0.$$

An equation of the third degree, which may be solved by HORNER'S method (BURNSIDE and PANTON 1886), or by the method of successive trials developed on page 532.

In the present case the solution is

$$\theta = 0.5902.$$

Then, assuming equal crossing over in male and female

$$p = \sqrt{\bar{\theta}} = 0.7682$$

or 23.18 percent crossing over, with coupling.

The variance of this estimate of  $\theta$  may be obtained by differentiating equation 2 again with respect to  $\theta$ , substituting the expected values for the class frequencies, and equating to  $-1/V(\theta)$

$$-\frac{3n}{16} \cdot \frac{2+\theta}{(2+\theta)^2} - \frac{3n}{16} \cdot \frac{1-\theta}{(1-\theta)^2} - \frac{3n}{16} \cdot \frac{2-\theta}{(2-\theta)^2} - \frac{9n}{16} \cdot \frac{1+3\theta}{(1+3\theta)^2} = -\frac{1}{V(\theta)}$$

or

$$\frac{1}{V(\theta)} = \frac{3n}{16} \left( \frac{1}{2+\theta} + \frac{1}{1+\theta} + \frac{1}{2-\theta} + \frac{3}{1+3\theta} \right) \quad (3)$$

$$V(\theta) = \frac{4}{3n} \cdot \frac{(2+\theta)(1-\theta)(2-\theta)(1+3\theta)}{5+2\theta-4\theta^2} \quad (4)$$

Then, since the variance of  $\theta$  is the mean square deviation of all  $\theta$ 's from the mean of  $\theta$ , and the variance of  $p$  is the mean square deviation of the  $p$ 's from the mean of  $p$ , and since  $\theta$  equals  $p^2$ , we can calculate the variance of  $p$  from the variance of  $\theta$ .

$$V(\theta) = (2p)^2 \cdot V(p)$$

and

$$V(p) = \frac{(2+p^2)(1-p^2)(2-p^2)(1+3p^2)}{3np^2(5+2p^2-4p^4)} \quad (5)$$

Substituting for  $p$  we get

$$V(p) = 0.0001240$$

$$\sigma(p) = 0.011$$

BRUNSON's formula may be reduced to

$$p_B^2 = \frac{16}{18n} \cdot (a-b-c+3d)$$

where  $a$ ,  $b$ ,  $c$ , and  $d$  are the observed frequencies in the four classes,  $CRP_{a1}$ ,  $CRp_{a1}$ ,  $[Cr+cR+cr]P_{a1}$ , and  $[Cr+cR+cr]p_{a1}$  respectively.

BRUNSON finds

$$p_B = 0.767.$$

The variance of this estimate may be found from the general equation given by FISHER (1928).

$$\frac{1}{n}V(T) = S\left\{p\left(\frac{dT}{da}\right)^2\right\} - \left(\frac{dT}{dn}\right)^2 \quad (6)$$

where  $T$  is any function of the frequencies, and  $p$  is the probability of the corresponding class  $a, b, c, d$ . For convenience  $T_B$  may be used in place of  $p_B$ , and the variance of  $p_B$  found from that of  $T_B$  as above.

Then

$$T_B = \frac{16}{18n}(a-b-c+3d).$$

Taking the components of

$$S\left\{p\left(\frac{dT}{da}\right)^2\right\}$$

$$\frac{dT}{da} = \frac{16}{18n} \quad ; \quad \left(\frac{dT}{da}\right)^2 = \frac{64}{81n^2}$$

$$\frac{dT}{db} = -\frac{16}{18n} \quad ; \quad \left(\frac{dT}{db}\right)^2 = \frac{64}{81n^2}$$

$$\frac{dT}{dc} = -\frac{16}{18n} \quad ; \quad \left(\frac{dT}{dc}\right)^2 = \frac{64}{81n^2}$$

$$\frac{dT}{dd} = \frac{48}{18n} \quad ; \quad \left(\frac{dT}{dd}\right)^2 = \frac{64}{9n^2}$$

and

$$\begin{aligned} S\left\{p\left(\frac{dT}{da}\right)^2\right\} &= (pa+pb+pc+9pd)\frac{64}{81n^2} \\ &= \frac{24(1+\theta)}{16} \times \frac{64}{81n^2} = \frac{32(1+\theta)}{27n^2}. \end{aligned}$$

And the component

$$\begin{aligned} \left(\frac{dT}{dn}\right)^2 &= \left(-\frac{16(a-b-c+3d)}{18n^2}\right)^2 \\ &= \left(\frac{-18n\theta}{18n^2}\right)^2 = \frac{\theta^2}{n^2} \end{aligned}$$

Then

$$\frac{1}{n}V(T_B) = \frac{32+32\theta-27\theta^2}{27n^2}$$

$$V(T_B) = \frac{32+32\theta-27\theta^2}{27n}$$



Then since

$$T_B = p_B^2 ; \quad V(p_B) = \frac{V(T_B)}{4/22}$$

as before, and

$$V(p_B) = \frac{32 + 32p^2 - 27p^4}{108np^2}.$$

Substituting the most likely value of  $p$  we get

$$V(p_B) = 0.000165$$

$$\sigma(p_B) = 0.013.$$

The efficiency of BRUNSON's formula may be estimated by dividing the variance of the Maximum Likelihood equation by the variance of BRUNSON's formula.

$$E = \frac{(2+p^2)(1-p^2)(2-p^2)(1+3p^2)}{3np^2(5+2p^2-4p^4)} \div \frac{32+32p^2-27p^4}{108np^2}$$

$$= \frac{36(2+p^2)(1-p^2)(2-p^2)(1+3p^2)}{(5+2p^2-4p^4)(32+32p^2-27p^4)}.$$

For the distribution of  $E$  for values of  $p$  from 0 to 1, see figure 1. The formula is about 90 percent efficiency throughout the repulsion phase, but compares badly with the Maximum Likelihood equation for high coupling. Nowhere does it give complete efficiency.

The expected frequencies may be found by substituting the most likely value of  $\theta$  in the probabilities and multiplying by  $n = 3496$ .

TABLE 2

*Comparison of observation with expectation, obtained by two different solutions (BRUNSON's data).*

		$CRp_{\theta_1}$	$CRp_{\theta_1}$	$(Cr+cR+c)r P_{\theta_1}$	$(Cr+cR+c)r p_{\theta_1}$	$n$
Observed		1907	300	1053	686	3946
Expected {	<i>M. L.</i>	1916.42	303.20	1043.08	683.30	3946.00
	<i>BR.</i>	1915	305	1044	682	3946

$\chi^2$  from Maximum Likelihood expectation = 0.185.

$\chi^2$  from Brunson's expectation = 0.2165.

In each case there are 2 degrees of freedom from which to determine  $P$ .<sup>1</sup>

<sup>1</sup> FISHER (1922, 1923 and 1924) has shown that when a population, with which a sample is to be compared, has itself been reconstructed from the sample, the distribution of  $\chi^2$  is not known simply from the number of frequency classes. When using ELDETON's tables, the table must be entered with  $n^1$  equal to one more than the number of degrees of freedom in which the sample may

Any number of formulae for the estimation of  $p$  may be invented. See FISHER (1928, in press).

The amount of information per plant obtained from the  $F_2$  and its corresponding backcross may be compared with the amount of information obtainable from a simple backcross, that is, one in which complete classification is possible.

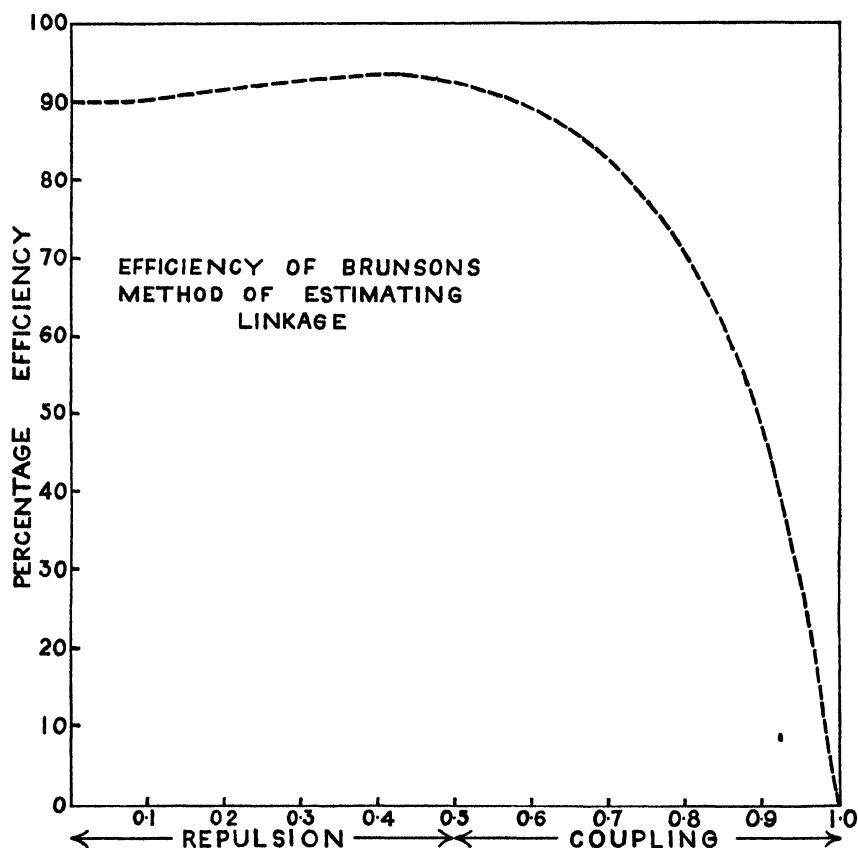


FIGURE 1.—Efficiency of BRUNSON'S method of estimating linkage in an  $F_2$  involving a factor linked to one of two complementary factors.

For a simple backcross:

$$V(p) = \frac{p(1-p)}{n}.$$

differ from the reconstructed population. Doctor FISHER (1928) gives a table of  $\chi^2$  which is more convenient for use than that given by ELDERTON, and in using FISHER'S table, the table is entered with  $n$  equal to the number of degrees of freedom in which the sample may differ from the reconstructed population.

Therefore the amount of information concerning  $p$  available per plant is

$$\frac{I(p)}{n} = \frac{1}{p(1-p)}.$$

In a backcross between an  $F_1$  of the type under consideration and its triple recessive, the probabilities of the four classes will be:

$$\begin{array}{cccc} CRP_{\sigma_1} & CRp_{\sigma_1} & (Cr+cR+cr)P_{\sigma_1} & (Cr+cR+cr)p_{\sigma_1} \\ \frac{p}{4} & \frac{1-p}{4} & \frac{2-p}{4} & \frac{1+p}{4} \end{array}$$

And the maximum likelihood value of  $p$  is that for which

$$\frac{a}{p} - \frac{b}{1-p} - \frac{c}{2-p} + \frac{d}{1+p} = 0$$

and the variance of  $p$

$$\begin{aligned} \frac{1}{V(p)} &= \frac{n}{4} \left( \frac{1}{p} + \frac{1}{1-p} + \frac{1}{2-p} + \frac{1}{1+p} \right) \\ V(p) &= \frac{2p(1-p)(2-p)(1+p)}{n(1+2p-2p^2)}. \end{aligned}$$

Then the amount of information concerning  $p$  available per plant is the reciprocal of the variance, divided by  $n$

$$\frac{I(p)}{n} = \frac{1+2p-2p^2}{2p(1-p)(2-p)(1+p)}.$$

The amount of information available per plant relative to that obtainable from a simple backcross is

$$\frac{1+2p-2p^2}{2p(1-p)(2-p)(1+p)} \div \frac{1}{p(1-p)} = \frac{1+2p-2p^2}{2(2-p)(1+p)}.$$

A similar procedure may be applied to the Maximum Likelihood solution for the  $F_2$  and to BRUNSON's formula. The amount of information concerning  $p$  made available per  $F_2$  plant by the Maximum Likelihood equation is

$$\frac{I(p)}{n} = \frac{3p^2(5+2p^2-4p^4)}{(2+p^2)(1+p)(1-p)(2-p^2)(1+3p^2)}.$$

Dividing by  $1/p(1-p)$  we get the amount of information per  $F_2$  plant relative to that supplied by a simple backcross

$$\frac{3p^2(5+2p^2-4p^4)}{(2+p^2)(1+p)(2-p^2)(1+3p^2)}.$$

The amount of information concerning  $p$  made available per  $F_2$  plant by BRUNSON'S formula is

$$\frac{I(p_B)}{n} = \frac{108p^3}{32+32p^2-27p^4}.$$

Dividing by  $1/p(1-p)$ , we get the amount of information per  $F_2$  plant relative to that supplied by a simple backcross

$$\frac{108p^3(1-p)}{32+32p^2-27p^4}.$$

These amounts of information are plotted as percentages for values of  $p$  from 0 to 1 in figure 2. It will be seen that for values of  $p$  from 0.7 to 1.0—

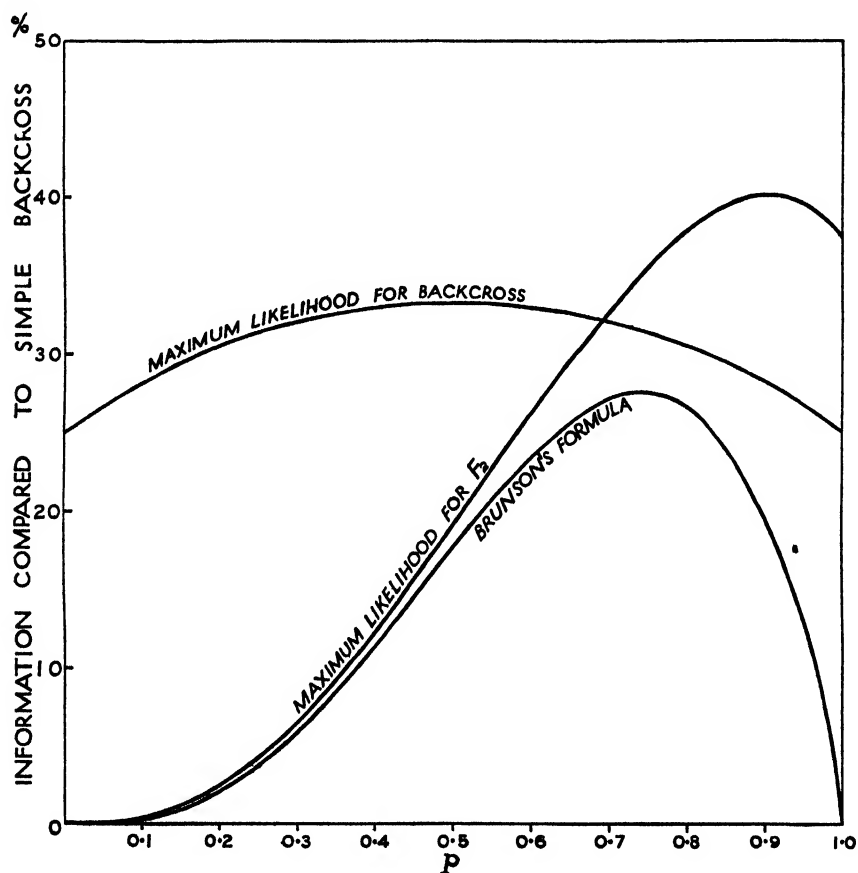


FIGURE 2.—A factor linked to one of two complementary factors: Amount of information concerning linkage supplied per plant by a backcross to a triple recessive, and by an  $F_2$  using (a) Maximum Likelihood solution, (b) Brunson's solution. Amount of information per plant expressed as a percentage of that supplied by a simple backcross.

that is, 30 percent or less crossing over with coupling—an  $F_2$  gives a better estimate of linkage than a backcross to the triple recessive. A simple backcross can, of course, be obtained by backcrossing to a double recessive, which is homozygous dominant for the independent complementary factor. In the present instance, backcrossing is impossible, because one of the linked factors is lethal in the recessive phase. In any case, it is not known which of the complementary factors is independent.

The amount of information supplied by the backcross is never more than  $1/3$  of what would be available if the classification could be completed.  $F_2$ s give very little information concerning linkage in the repulsion phase, and at best, in the coupling phase, give 40 percent of the information that would be supplied by a simple backcross of the same size.

#### A CASE INVOLVING DUPLICATE FACTORS

WOODWORTH (1921) has studied a case in soy beans in which one of two duplicate factors,  $I$  and  $D$ , for cotyledon colour is linked with a factor  $V$  for seed coat colour, and gives the figures given in table 3 for his  $F_2$ , after correcting his data to 15:1 for cotyledon colour. This correction is made necessary because seeds borne on the  $F_1$  plants were classified for cotyledon colour, while seeds borne on  $F_2$  plants were classified for seed coat colour.

TABLE 3

$F_2$  distribution for cotyledon colour (corrected to 15:1 nearest whole number) and seed coat colour (WOODWORTH'S data).

	$ID+Id+iD$	$id$	COAT COLOUR
$V$	150	14	164
$v$	64	0	64
Cotyledon colour	214	14	$n=228$

The probabilities of the four classes are given in table 4, where  $\theta$  expresses the effect of the linkage, and, as before, is equal to  $p^2$  if crossing over is the same in male and female.

TABLE 4

Probabilities of the four classes where  $\theta$  expresses the linkage between either  $I$  or  $D$ , and  $V$ .

	$ID+Id+iD$	$id$	COAT COLOUR
$V$	$\frac{11+\theta}{16}$	$\frac{1-\theta}{16}$	$\frac{3}{4}$
$v$	$\frac{4-\theta}{16}$	$\frac{\theta}{16}$	$\frac{1}{4}$
Cotyledon colour	$\frac{15}{16}$	$\frac{1}{16}$	$n=1$

Then the most likely value of  $\theta$  is that for which the first differential of the likelihood equation is 0,

$$\frac{150}{11+\theta} - \frac{64}{4-\theta} - \frac{14}{1-\theta} + \frac{0}{\theta} = 0.$$

Then, clearly, the most likely value of  $\theta$  is 0,—that is, complete linkage with repulsion.

The variance of this estimate is obtained, as before, by differentiating again, substituting the probabilities for the observed frequencies, and equating to  $-1/V(\theta)$

$$\frac{1}{V(\theta)} = \frac{n}{16} \left\{ \frac{1}{11+\theta} + \frac{1}{4-\theta} + \frac{1}{1-\theta} + \frac{1}{\theta} \right\}$$

$$V(\theta) = \frac{4(11+\theta)(4-\theta)(1-\theta)\theta}{n(11+2\theta-4\theta^2)}.$$

Then, since  $\theta = p^2$  we can derive the variance of  $p$  as before.

$$V(p) = \frac{(11+p^2)(4-p^2)(1-p^2)}{n(11+2p^2-4p^4)}.$$

Substituting  $\theta = 0$  we get

$$V(p) = \frac{44}{11 \times 228} = 0.01754$$

$$\sigma(p) = 0.132.$$

The  $F_2$  evidence indicates, therefore, 0 percent crossing over, with a standard deviation of 13.2 percent. WOODWORTH (1921 and 1923) gives equations<sup>2</sup> for the estimation of linkage, which may be reduced to:

$$p_w^2 = \theta_w = \frac{a+b-15c+d}{n}.$$

On applying this to the corrected data, WOODWORTH gets 13.25 percent crossing over. This, however, he shows to be due to the fact that the correction is to the nearest whole number, only, and when the exact corrected figures are taken, there is no evidence of crossing over.

The variance of  $\theta_w$  may be obtained by applying equation 6 as before.

<sup>2</sup> WOODWORTH (1921) gives:  $r = .25\sqrt{a+b+d+15c}$ .

This is clearly an error for  $r = .25\sqrt{a+b+d-15c}$ .

$$\begin{aligned}
 S\left\{p\left(\frac{d\theta w}{da}\right)^2\right\} &= \frac{11+\theta}{16} \times \frac{1}{n^2} + \frac{4-\theta}{16} \times \frac{1}{n^2} + \frac{1-\theta}{16} \times \frac{225}{n^2} + \frac{\theta}{16} \times \frac{1}{n^2} \\
 &= \frac{15-14\theta}{n^2} \\
 \left(\frac{d\theta}{dn}\right)^2 &= \left(\frac{-a-b+15c-d}{n^2}\right)^2 \\
 &= \left(\frac{-16n\theta}{16n^2}\right)^2 \\
 &= \frac{\theta^2}{n^2}.
 \end{aligned}$$

Then

$$\frac{1}{n}V(\theta_w) = \frac{15-14\theta-\theta^2}{n^2}$$

Then, since  $\theta_w = p_w^2$ , as before, the variance of  $p_w$  will be

$$V(p_w) = \frac{15-14p^2-p^4}{4np^2}.$$

Then, since  $p=0$ , in this case the variance of WOODWORTH'S estimate is infinitely large.

The expected numbers are obtained by substituting  $\theta=0$  in the probabilities, and multiplying by  $n=228$ . Having corrected the ratio for cotyledon colour, and fitted an estimate of  $p$ , only 1 degree of freedom remains, and  $P$  = about 0.28. (See footnote to page 523.)

TABLE 5

Observed and expected frequencies for cotyledon colour and seed coat colour in soy beans.  $\chi^2=1.1548$ .

	$[ID+Id+iD]V$	$[ID+Id+iD]v$	$idV$	$idv$	$n$
Observed	150	64	14	0	228
Expected	155.75	57.00	14.25	0.00	228
Deviations	-6.75	+7.0	-0.25	0	

The probabilities of the classes in a backcross involving duplicate genes, one of which is linked to a third gene, will be

$$\begin{aligned}
 &[ID+Id+iD]V : [ID+Id+iD]v : idV : idv \\
 &\frac{1+p}{4} \qquad \frac{2-p}{4} \qquad \frac{1-p}{4} \quad \frac{p}{4}
 \end{aligned}$$

and the variance of  $p$  will be

$$V(p) = \frac{2p(1-p)(2-p)(1+p)}{n(1+2p-2p^2)}$$

the same as for complementary factors.

The amounts of information concerning  $p$  available per plant, compared to a simple backcross are calculated as before and give

Maximum Likelihood equation for  $F_2$

$$\frac{p(11+2p^2-4p^4)}{4(11+p^2)(4-p^2)(1+p)}$$

WOODWORTH'S formula for  $F_2$

$$\frac{4p^2(1-p)}{15-14p^2-p^4}$$

Maximum Likelihood equation for backcross

$$\frac{(1+2p-2p^2)}{2(2-p)(1+p)}$$

These amounts of information are plotted as percentages of that obtainable from a simple backcross in figure 3. It is quite clear that very little information concerning linkage can be obtained from an  $F_2$  involving duplicate factors under any circumstances, and such  $F_2$ s should always be carried on to  $F_3$ . A backcross to the triple recessive gives from 1/4 to 1/3 of the information per plant that would be obtained from a simple backcross.

TABLE 6

*Soy bean  $F_2$  segregating for seed coat colour and cotyledon colour: yellow cotyledon plants only included and these analysed according to  $F_3$  behaviour (WOODWORTH'S data).*

	TRUE BREEDING YELLOWS	SPLITTING YELLOWS		COAT COLOUR
		Double Het.	Single Het.	
$V$	57	45	45	147
$v$	55	3	5	63
Cotyledon colour	112	48	50	$n = 210$

WOODWORTH is able to classify the 210 plants which came from embryos having yellow cotyledons into: (1) plants homozygous dominant for  $I$  or  $D$  or both; (2) plants segregating for both  $I$  and  $D$ ; and (3) plants homozygous recessive for either  $I$  or  $D$  and segregating for either  $D$  or  $I$ . (See table 6.) Then the probabilities of the classes will be as given in table 7.



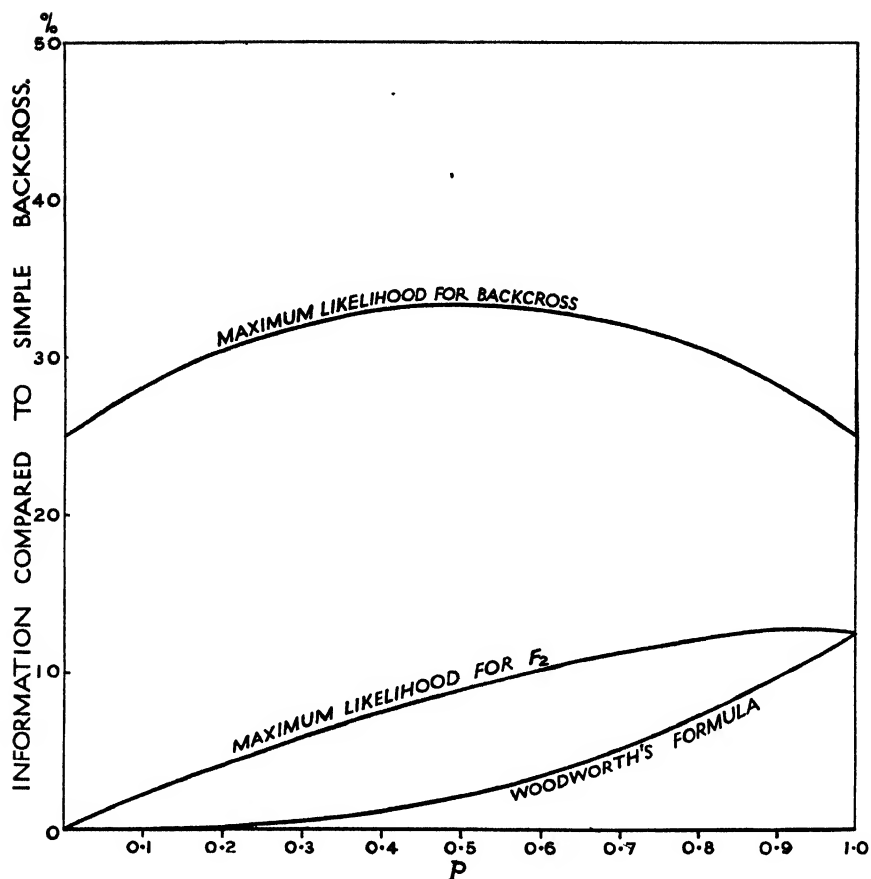


FIGURE 3.—A factor linked to one of two duplicate factors: Amount of information concerning linkage supplied per plant by a backcross to a triple recessive, and by an  $F_2$ , using (a) Maximum Likelihood solution, (b) Woodworth's solution. Amount of information per plant expressed as a percentage of that supplied by a simple backcross.

TABLE 7

*Probabilities of the classes involved in the soy bean segregation tabulated in table 6.*

	TRUE BREEDING YELLOWS	SPLITTING YELLOWS		COAT COLOUR
		Double Het.	Single Het.	
$V$	$\frac{3}{15}(1+2p-p^2)$	$\frac{4}{15}(1-p+p^2)$	$\frac{2}{15}(2-p)$	$\frac{11+p^2}{15}$
$v$	$\frac{1}{15}(4-6p+3p^2)$	$\frac{4}{15}p(1-p)$	$\frac{2p}{15}$	$\frac{4-p^2}{15}$
Cotyledon colour	$\frac{7}{15}$	$\frac{4}{15}$	$\frac{4}{15}$	$n=1$

Then the garitholm of the likelihood will be

$$L = 57 \log \frac{3}{15}(1+2p-p^2) + 45 \log \frac{4}{15}(1-p+p^2) + 45 \log \frac{2}{15}(2-p) \\ + 55 \log \frac{4-6p+3p^2}{15} + 3 \log \frac{4p}{15}(1-p) + 5 \log \frac{2p}{15}.$$

And the most likely value of  $p$  will be that for which

$$\frac{114(1-p)}{1+2p-p^2} - \frac{45(1-2p)}{1-p+p^2} - \frac{45}{2-p} - \frac{330(1-p)}{4-6p+3p^2} + \frac{8}{p} - \frac{3}{1-p} = x = 0.$$

This equation may be multiplied up and solved by HORNER's method, but an easier method is to obtain the value of  $p$  by successive trials. This method has the advantage that it avoids all algebraic manipulation, and provides not only an estimate of the linkage, but also an estimate of its standard error. A good guess for the first trial can usually be made after inspecting the data. In this example the  $F_2$  indicated no crossing over, while the data in table 6 show that repulsion is not complete. A fair first trial is therefore  $p = 0.1$ . Since the amount of information  $[=1/V(p)]$  is the rate of change of  $x$  relative to  $p$ , the two final approximations, if worked out to sufficient accuracy, will give an estimate of the variance of  $p$ . The two final approximations used were  $p = 0.144$  and  $p = 0.115$ .

When

$$p = 0.114 \quad x = +0.26904$$

$$p = 0.115 \quad x = -0.54503.$$

Therefore  $p$  is approximately 0.1143, or 11.43 percent crossing over. And for a change of 0.001 in  $p$ ,  $x$  changes 0.81407, and the rate of change of  $p = 1/V(p) = 814.07$

$$V(p) = 0.001228$$

$$\sigma(p) = 0.03504.$$

This may be checked by differentiating the likelihood equation a second time, equating to  $-1/V(p)$  and substituting  $p = 0.11433$

$$\frac{1}{V(p)} = \frac{114(3-2p+p^2)}{(1+2p-p^2)^2} - \frac{45(1+2p-2p^2)}{(1-p+p^2)^2} + \frac{45}{(2-p)^2} + \frac{330(2-6p+3p^2)}{(4-6p+3p^2)^2} \\ + \frac{8}{p^2} + \frac{3}{(1-p)^2} = 816.05$$

$$V(p) = 0.001225$$

$$\sigma(p) = 0.03500.$$

The two estimates of the variance differ by less than 0.5 percent.

We have, therefore, 11.43 percent crossing over with a standard deviation of 3.5 percent.

#### CONSIDERATION OF THEORY WHEN COMPLEMENTARY OR DUPLICATE FACTORS ARE THEMSELVES LINKED

These cases are rare, but may be considered for the sake of completeness.

##### (a) *Complementary Factors*

The probabilities of the two classes will be

$$\frac{2+\theta}{4} : \frac{2-\theta}{4}$$

where, as before,  $\theta = p^2$  if crossing over is the same in male and female.

The most likely value of  $\theta$  will be that for which

$$\frac{a}{2+\theta} - \frac{b}{2-\theta} = 0$$

or

$$p^2 = \theta = \frac{2(a-b)}{n}$$

And

$$\frac{1}{V(\theta)} = \frac{n}{4} \left( \frac{1}{2+\theta} + \frac{1}{2-\theta} \right)$$

$$V(\theta) = \frac{(2+\theta)(2-\theta)}{n}$$

And since  $\theta = p^2$ , we calculate the variance of  $p$  as before

$$V(p) = \frac{(2+p^2)(2-p^2)}{4p^2n}$$

And the amount of information per plant compared to a (theoretical) simple backcross will be the reciprocal of this divided by  $n/p(1-p)$

$$\frac{4p^3(1-p)}{4-p^4}$$

##### (b) *Duplicate Factors*

The probabilities of the two classes will be

$$\frac{4-\theta}{4} : \frac{\theta}{4}$$

The most likely value of  $\theta$  will be that for which

$$\frac{b}{\theta} - \frac{a}{4-\theta} = 0$$

or

$$p^2 = \theta = \frac{4b}{n}.$$

And differentiating again, substituting probabilities for  $a$  and  $b$  and equating to  $-1/V(\theta)$  we get

$$\frac{1}{V(\theta)} = \frac{n}{4} \left\{ \frac{1}{\theta} + \frac{1}{4-\theta} \right\}$$

$$V(\theta) = \frac{\theta(4-\theta)}{n}$$

$$V(p) = \frac{4-p^2}{4n}.$$

And the amount of information per plant compared with a simple back-cross will be

$$\frac{4p(1-p)}{4-p^2}.$$

The probabilities of the classes in the two backcrosses will be

$$\text{Complementary } \frac{p}{2}[AB] : \frac{2-p}{2}[Ab+aB+ab]$$

$$\text{Duplicate } \frac{2-p}{2}[AB+Ab+aB] : \frac{p}{2}ab.$$

And the most likely values of  $p$  will be of those for which

$$\frac{a}{p} - \frac{b}{2-p} = 0 \text{ for Complementary Factors.}$$

and

$$\frac{b}{p} - \frac{a}{2-p} = 0 \text{ for Duplicate Factors.}$$

The variance of  $p$  is the same in each case:

$$\begin{aligned} \frac{1}{V(p)} &= \frac{n}{2} \left( \frac{1}{p} + \frac{1}{2-p} \right) \\ &= \frac{n}{p(2-p)} \end{aligned}$$

$$V(p) = \frac{p(2-p)}{n}$$

Then the amount of information per plant relative to a simple backcross will be the reciprocal of the variance divided by  $n/p(1-p)$

$$= \frac{1-p}{2-p}.$$

These amounts of information are plotted as percentages of the amount of information supplied by a simple backcross for values of  $p$  from 0 to 1. See figure 4.

Very little information concerning linkage can be obtained from an  $F_2$  involving linked complementary factors. Where there is a considerable

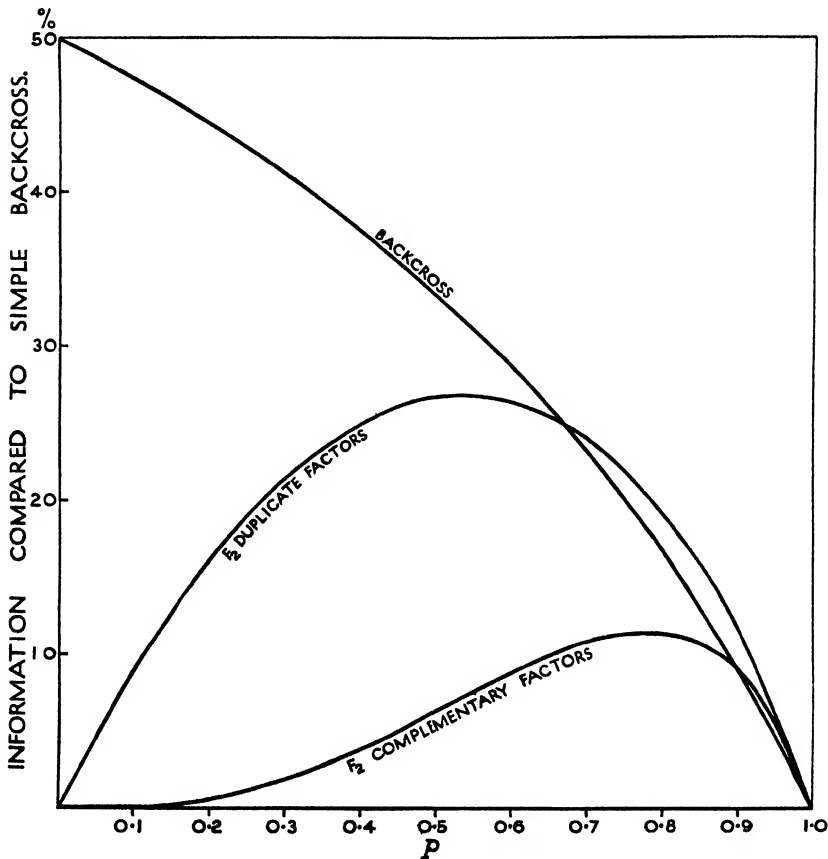


FIGURE 4.—Complementary factors themselves linked, and duplicate factors themselves linked. Amount of information concerning linkage supplied per plant by a backcross to a double recessive, and by  $F_2$  using Maximum Likelihood solution. Amount of information per plant expressed as a percentage of that supplied by a simple backcross.

amount of crossing over—that is, where  $p$  is near 0.5—an  $F_2$  involving linked duplicate factors will give about  $1/4$  of the information that would be given by a simple backcross of the same size. The backcrosses give up to  $1/2$  of the information obtainable from a simple backcross with high linkage in the repulsion phase, but become less efficient than the corresponding  $F_2$ s with high coupling.

#### SUMMARY

1. The “Method of Maximum Likelihood” developed by DOCTOR R. A. FISHER is applied to the problem of estimating linkage in cases involving complementary and duplicate factors.

2. Variances are calculated for existing formulae, and their efficiencies are determined to show that the “Method of Maximum Likelihood” is in all cases superior to any other method of estimation.

3. The amount of information supplied per plant by Maximum Likelihood formulae for  $F_2$ s and backcrosses, and by other formulae for  $F_2$ s is calculated and compared with the amount of information supplied per plant by a simple—that is, completely classified—backcross. (See figures 2, 3 and 4.) From these curves it is possible to estimate the size of family necessary to give any required degree of accuracy.

#### ACKNOWLEDGMENTS

The investigation here presented was carried out at ROTHAMSTED EXPERIMENTAL STATION under the guidance of DOCTOR R. A. FISHER, during a period of study leave from the Genetics Department of the COTTON RESEARCH STATION, Trinidad, B. W. I. The author is indebted to the EMPIRE COTTON GROWING CORPORATION for granting the leave, and to DOCTOR R. A. FISHER for suggesting the problem, and for the care and patience with which he supervised both its general development, and its detailed application.

Since the above was written a paper on the calculation of linkage intensities by F. V. OWEN has come under notice. FISHER's Method of Maximum Likelihood is considered, but its particular virtue, namely, that, in the theory of large samples, no statistic can have a smaller sampling variance, is overlooked; see FISHER (1928).

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XVII.—The Distribution of Gene Ratios for Rare Mutations. By  
R. A. Fisher, Sc.D., F.R.S. (Rothamsted Experimental Station,  
Harpenden, Herts). *Communicated by Professor GODFREY H.*  
THOMSON.

(MS. received March 21, 1930. Read May 5, 1930.)

1. INTRODUCTORY.

IN 1922 the author published a short paper, "On the Dominance Ratio," in the *Proceedings of the Royal Society of Edinburgh* (vol. xlii, pp. 321-341). Among other results, the conclusion was drawn that in the total absence of mutations and of selective survival, the quantity of variation, the variance, of an interbreeding group would decrease by reason of random survival, at a rate such that the "time of relaxation" was  $4n$  generations, where  $n$  is the number breeding in each generation.

The variance after the lapse of  $T$  generations was found to be proportional to  $e^{-T/4n}$ .

During last year Professor Sewall Wright of Chicago has been good enough to send me in MS. an investigation in which, while confirming many other conclusions of my paper, he arrives at a time of relaxation of only  $2n$  generations. Both periods are in most species so enormous that they lead to the same conclusion, namely, that random survival, while of great importance in conditioning the fate of an individual mutant gene, is a totally unimportant factor in the balance of forces by which the actual variability of species is determined. Nevertheless it will, I hope, minimise the confusion which every error is liable to cause if I put on record at once my acceptance of Professor Wright's value, and at the same time eradicate the error of my previous work by giving a more rigorous and comprehensive treatment of the whole subject. I may say that the previous conclusions as to the interpretation of the evidence for Mendelian dominance in the factors contributing to human variability are untouched, but that the rôle of mutations in maintaining the current genetic variability of a species may now be set in a much clearer light.

The error to be corrected lies in the derivation (p. 326) of the differential equation satisfied by the distribution of the frequency ratios of different factors, when none are subject to selective action. If the

two alternative genes in any locus appear in the ratio  $p : q$ , the variance of  $p$  after one generation of random breeding will be

$$\frac{pq}{2n},$$

where  $n$  is the number breeding in each generation. To avoid the inconvenience that this variance is a function of  $p$ , we may write

$$2p = 1 - \cos \theta, \quad 2q = 1 + \cos \theta$$

when

$$\delta p = \sqrt{pq} \delta \theta$$

and the variance of  $\theta$  is therefore very nearly constant at the value  $1/2n$ .

Although,  $n$  being large, the values of  $\theta$  after one generation of random breeding will be well represented by a normal distribution with constant variance, yet its mean will differ from zero by an amount of under  $1/n$ . This was overlooked in the previous treatment; to find the mean of  $\delta \theta$  as far as terms in  $n^{-1}$ , we may write

$$\delta \theta = \frac{1}{\sqrt{pq}} \delta p - \frac{1-2p}{4pq\sqrt{pq}} (\delta p)^2 \dots,$$

then since the mean value of  $\delta p$  is strictly zero, while that of  $(\delta p)^2$  is  $pq/2n$ , the mean value of  $\delta \theta$  is seen to be

$$-\frac{1-2p}{8n\sqrt{pq}} = -\frac{1}{4n} \cot \theta.$$

This, of course, with values of  $n$  of many millions, is an exceedingly small quantity, but its effect is not negligible for the discussion required, for if

$$df = y d\theta$$

is the distribution of the values of  $\theta$  for different factors, the flux past every value of  $\theta$  due to random reproduction in one generation is changed from

$$-\frac{1}{4n} \frac{\partial y}{\partial \theta}$$

to

$$-\frac{y}{4n} \cot \theta - \frac{1}{4n} \frac{\partial y}{\partial \theta},$$

and the differential equation to be satisfied by  $y$  becomes

$$\frac{\partial y}{\partial T} = \frac{1}{4n} \left\{ \frac{\partial}{\partial \theta} (y \cot \theta) + \frac{\partial^2 y}{\partial \theta^2} \right\}, \quad (1)$$

instead of

$$\frac{\partial y}{\partial T} = \frac{1}{4n} \frac{\partial^2 y}{\partial \theta^2},$$

the equation previously obtained; in both  $T$  is measured in generations.

## 2. THE SOLUTION FOR STEADY DECAY.

It so happens that the function of  $\theta$  which satisfies the true equation in the case when, in the absence of mutations, the variance is steadily decaying owing to chance extinctions at the termini  $\theta=0$ ,  $\theta=\pi$ , is the same as the corresponding solution of the original erroneous equation, namely,  $y=A \sin \theta$ .

Substituting in the true equation we have

$$\frac{\partial A}{\partial T} \sin \theta = \frac{2A \sin \theta}{4n},$$

or

$$A = A_0 e^{-T/2n},$$

in place of

$$A = A_0 e^{-T/4n}$$

originally obtained. This confirms the value of  $2n$  generations for the time of relaxation, found by a quite independent method by Professor Wright. The variance will then be halved by random survival in  $2n \log 2 = 1.4n$  generations. The immense length of this period for most species shows how trifling a part random survival must play in the balance of influences which determines the actual variability.

## 3. VARIABILITY MAINTAINED CONSTANT BY MUTATIONS IN THE ABSENCE OF SELECTION.

If in equation (1) we put  $\partial y/\partial T$  equal to zero, we may at once integrate the right-hand side in the form

$$\frac{\partial y}{\partial \theta} + y \cot \theta = -4nB,$$

where  $B$  is the net number of factors in each generation, the gene ratios of which flow past any specified value of  $\theta$ , and the differential equation now simply represents the fact that this flux is the same for all values of  $\theta$ . The equation may now be integrated giving the primitive,

$$y \sin \theta = A + 4nB \cos \theta$$

or

$$y = A \operatorname{cosec} \theta + 4nB \cot \theta. \quad (2)$$

If we make the convention that mutations are equally frequent in supplying factors with  $\theta$  near to zero and in supplying factors with  $\theta$  near to  $\pi$ , the symmetrical solution

$$y = A \operatorname{cosec} \theta$$

will be appropriate; but, if we suppose all mutations occur at  $\theta=0$ , then  $y$  should tend to zero at  $\theta=\pi$ , and the appropriate form is

$$y = 4nB(\operatorname{cosec} \theta + \cot \theta). \quad (3)$$

In either case the integral of  $y$  to the limit of its range at  $\theta=0$  fails to converge, so that the relation between the number of factors maintained and the rate of mutation cannot be made out without an investigation of the terminal conditions. Before passing on to consider

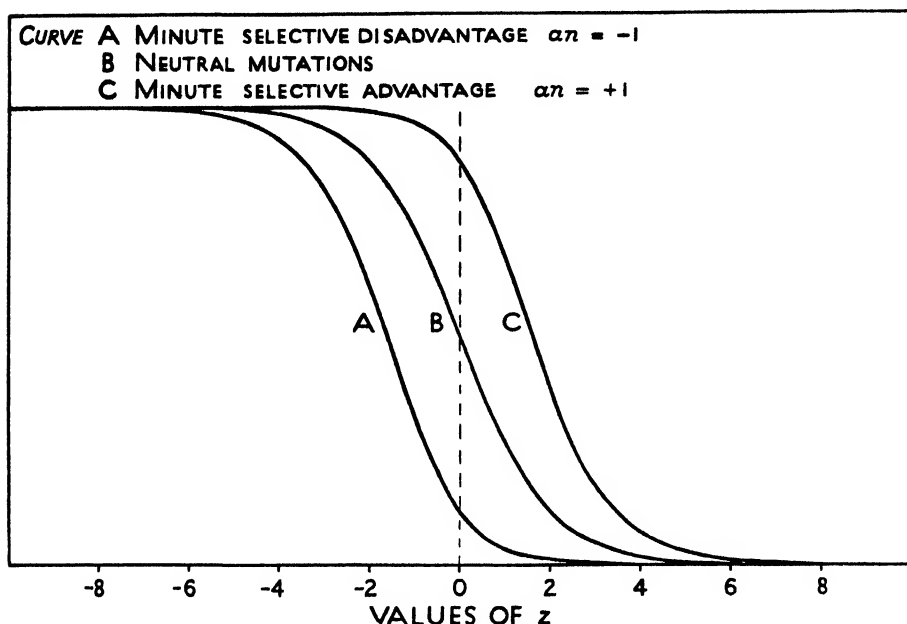


FIG. 1.—Frequency curves of logarithmic gene ratio,  $z$ , for different levels of selective advantage; note that the frequency ordinate is highest for the most extreme admissible negative values of  $z$ , and remains nearly constant over a range which is extremely sensitive to small selective intensities.

these, we may consider the distribution now obtained as a distribution not of  $\theta$  but of the more convenient variate  $z = \log(p/q)$ , the logarithmic gene ratio. The frequency distribution (3) may be represented on the scale of  $z$  by noting that

$$\begin{aligned} df &= 4nB (\operatorname{cosec} \theta + \cot \theta) d\theta \\ &= 4nB q dz \\ &= 4nB \frac{dz}{1 + e^z}. \end{aligned}$$

This frequency distribution is illustrated by curve B in fig. 1. The frequency ordinate is nearly constant for values of  $z$  less than  $-4$ , at

which point the mutant gene occupies nearly 2 per cent. of the available loci; it falls to half its previous value when  $z$  is raised to zero, when 50 per cent. of the loci are occupied by mutant genes. For higher gene ratios still, the frequency falls rather rapidly to zero. Since the frequency ordinate is nearly constant for high negative values of  $z$ , the total frequency maintained depends on how far the curve may be carried to the left, or how large (negative) values the logarithmic frequency ratio,  $z$ , may have. Evidently this will depend on the size of the population, and an exact treatment will evidently require an examination of the terminal conditions.

#### 4. DISTRIBUTIONS EXPRESSED BY FUNCTIONAL EQUATIONS.

A very powerful method of approach was indicated, but not utilised, in the previous paper. If

$$p_0, p_1, p_2, \dots$$

are the probabilities of an individual gene carried by a member of the species, being represented in the next generation in 0, 1, 2, . . . offspring, we may define a function

$$f(x) = p_0 + p_1x + p_2x^2 + \dots,$$

for values of  $x$  between 0 and 1, and it has been shown that to consider the offspring of two individuals instead of one, we have only to substitute

$$\{f(x)\}^2 \text{ for } f(x).$$

Consequently, if the number of factors in which the rarer gene occupies 1, 2, 3, . . . loci are given by  $\pi_1, \pi_2, \pi_3, \dots$ , and if

$$\phi(x) = \pi_1x + \pi_2x^2 + \pi_3x^3 + \dots,$$

the effect upon  $\phi$  of random breeding for one generation is to substitute

$$\phi\{f(x)\} \text{ for } \phi(x).$$

In practice we shall require to use the form

$$f(x) = e^{x-1},$$

and if we first take the case of extinction of genes without mutation, the distribution of gene frequencies, which maintains its form, while one factor is extinguished in each generation must satisfy the functional equation

$$\phi(e^{x-1}) = \phi(x) + \frac{1}{2},$$

for the distribution being symmetrical, half the extinctions may be taken to be reductions from 1, 2, 3, . . . loci to zero, and half to be increases to  $2n$ .

The corresponding equation for the generating function  $\phi$ , for the case of a distribution in equilibrium with mutations at the rate of one in each generation, is

$$\phi(e^{x-1}) - \phi(x) = 1 - x,$$

for a mutation may be represented as an increase of unity in the number of genes occupying one locus only, and a corresponding decrease of the (indefinite) number occupying no loci.

The solutions of these equations will be shown to correspond with the solutions of the differential equations obtained above, and to admit in addition of an investigation of the terminal condition.

### 5. THE FUNCTION $u_\nu$ .

In order to solve the functional equations, we define a function  $u_\nu$  of a single real variable  $\nu$ , which shall satisfy the condition

$$u_{\nu+1} = e^{u_\nu - 1},$$

starting from the arbitrary value  $u_0 = 0$ . The values of  $u_1, u_2, \dots$  may now be obtained by direct substitution, and these evidently tend to unity as a limit. To obtain a form for large values of  $\nu$ , we may put

$$v_\nu = \frac{1}{1 - u_\nu}$$

and obtain

$$v_{\nu+1} = 1 - \frac{1}{e^{1/v_\nu}} = v_\nu + \frac{1}{2} + \frac{1}{12v_\nu} - \frac{1}{720v_\nu^3} + \frac{B_6}{6! v_\nu^5} + \dots$$

Where  $B_6$ , etc., stand for the Bernoulli numbers

$$B_6 = \frac{1}{42} \quad B_8 = -\frac{1}{30} \quad B_{10} = \frac{5}{66} \quad B_{12} = -\frac{691}{2730}.$$

It appears from the recurrence formula of  $v$  that when  $v$  is large a first approximation is given by

$$v = \frac{1}{2},$$

and substituting this in the third term of the expression, we obtain the second approximation

$$v = \frac{1}{2}v + \frac{1}{8} \log v,$$

the error of which will tend to a finite limit, as  $v$  tends to infinity. Equally

$$\frac{1}{2}v - v + \frac{1}{8} \log v$$

must tend to a constant value as  $v$  is increased indefinitely. Let  $-C$  stand for this constant, and let

$$w_v = \frac{1}{2}v - v + \frac{1}{8} \log v + C,$$

then we may obtain an expansion for  $w$  in inverse powers of  $v$ ; for the recurrence formula provides that

$$w_{v+1} - w_v = \frac{1}{2} - \left\{ \frac{1}{2} + \frac{1}{12v} - \frac{1}{720v^3} + \dots \right\} + \frac{1}{6} \log \left\{ 1 + \frac{1}{2v} + \frac{1}{12v^2} - \frac{1}{720v^4} + \dots \right\}$$

and expanding this expression we obtain, dropping the suffix of  $v$ ,

$$\frac{v^{-2}}{144} + \frac{v^{-3}}{720} + \frac{v^{-4}}{24 \cdot 720} - \frac{v^{-5}}{42 \cdot 720} - \frac{v^{-6}}{1512 \cdot 720} + \frac{v^{-7}}{1680 \cdot 720} + \frac{1473v^{-8}}{336 \cdot 720^2} - \frac{v^{-9}}{924 \cdot 72 \cdot 720}.$$

as an expansion of  $w_{v+1} - w_v$ ; the first term shows that the leading term in the expansion of  $w$  is  $1/72v$  for

$$\frac{1}{v_{v+1}} - \frac{1}{v_v} = -\frac{1}{2v^2} + \frac{1}{6v^3} - \frac{1}{24v^4} + \dots,$$

and similar expansions may be obtained for  $(v_{v+1}^{-2} - v_v^{-2})$ , and so on. We thus obtain

$$w = \frac{v^{-1}}{72} + \frac{v^{-2}}{1080} - \frac{v^{-3}}{108 \cdot 144} - \frac{71v^{-4}}{168 \cdot 72^2} - \frac{8759v^{-5}}{630 \cdot 720^2} + \frac{31v^{-6}}{81 \cdot 720^2} + \frac{1637v^{-7}}{1008 \cdot 720^2} - \frac{20879093v^{-8}}{9504 \cdot 840 \cdot 720^2}.$$

While the last three coefficients are all less than  $10^{-5}$ , they show no such a decided tendency to decrease as would justify our evaluating the constant  $C$  by putting  $v=1$ ,  $v=0$ , a substitution which shows  $C$  to exceed by unity the limit of the sum of the coefficients. We may, however, use the larger values of  $v$  found by the recurrence formula for somewhat higher integral values of  $v$ .

For example, at  $v=5$ , the last three terms in  $w$  are less than  $10^{-9}$ , so that  $w$  will not be much in error in the ninth place of decimals;  $u$  is found by direct substitution in the recurrence formula to be .73192 31844 and

$$v - \frac{1}{8} \log v - \frac{1}{2}v + w = 1.01464,8607$$

gives a value of  $C$  nearly right to the last figure. To improve much upon this, it would be necessary to work to more than 10 places in the calculation of  $u$ . As a check, working to 14 places up to  $u_{10}$ , where the last term retained in  $w$  is about  $2 \times 10^{-12}$ , the value was found to be 1.01464 86071 7, a value which shows that the apparent precision attained by the series is not illusory.

## 6. SOLUTIONS OF THE FUNCTIONAL EQUATIONS.

If, in the equation

$$\phi(e^{2x-1}) - \phi(x) = \frac{1}{2},$$

we substitute

$$x = u,$$

we have

$$\phi(u_{\nu+1}) - \phi(u_\nu) = \frac{1}{2},$$

which is satisfied if  $\phi$  is the same function of  $x$  as  $\frac{1}{2}\nu$  is of  $u$ . But we know that

$$\frac{1}{2}\nu = \frac{1}{1-u} + \frac{1}{6} \log(1-u) - C + \frac{1-u}{72} + \frac{(1-u)^2}{1080} + \dots;$$

hence, apart from a finite fraction of the frequency,  $\phi$  may be expanded in powers of  $x$  in the form

$$\left. \begin{aligned} \phi(x) &= \frac{x}{1-x} + \frac{1}{6} \log(1-x) \\ &= \frac{5}{6}x + \frac{11}{12}x^2 + \frac{17}{18}x^3 + \dots \end{aligned} \right\}, \quad \dots \quad (4)$$

showing that in the distribution of gene ratios appropriate to steady extinction without mutation or selection, the frequency of factors represented in  $k$  loci must, when  $k$  is large, tend to unity. Since each step increases the gene proportion  $p$  by  $1/2n$ , we have, apart from the extremes of the distribution,

$$\begin{aligned} df &= 2ndp \\ &= n \sin \theta / \theta, \end{aligned}$$

in agreement with the solution obtained for this case from the differential equation. The total number of factors at all frequencies will be

$$2n - \frac{1}{2}(\gamma + \log 2n) - 0.1464, 86071, 7,$$

(where  $\gamma$  is Euler's constant 0.577215664), the remainder of which is negligible compared with the first term, twice the number of individuals breeding in each generation, thus verifying the rate of decay to be 1 in  $2n$  in each generation.

The exact treatment of the terminal frequencies, which shall account for the distribution of the finite quantity 0.014649, omitted from expression (4), evidently requires the differential coefficients of  $\frac{1}{2}\nu$  with respect to  $u$ , at the value  $u=0$ . Since the series for  $w$  in powers of  $(1-u)$  is itself doubtfully convergent at this value, its differential coefficients may be still less relied upon to converge; we therefore require reduction formulæ for these coefficients.

From the recurrence formula

$$u_\nu - 1 = \log u_{\nu+1},$$



we have, differentiating with respect to  $\nu$ ,

$$\frac{du_\nu}{d\nu} = \frac{1}{u_{\nu+1}} \frac{du_{\nu+1}}{d\nu}$$

or

$$\frac{d\nu}{du_\nu} = u_{\nu+1} \frac{d\nu}{du_{\nu+1}},$$

from which the value of  $d\nu/du$  for a lower value can be obtained with the same relative precision as at the higher.

We may write the relation in the form

$$\nu_0^I = u_1 \nu_1^I$$

with the understanding that any suffixes differing by unity can be substituted for those indicated. Since also

$$\frac{d}{du_0} = u_1 \frac{d}{du_1}$$

we can at once derive the further relations

$$\begin{aligned} \nu_0^{II} &= u_1 \nu_1^I + u_1^2 \nu_1^{II} \\ \nu_0^{III} &= u_1 \nu_1^I + 3u_1^2 \nu_1^{II} + u_1^3 \nu_1^{III} \\ \nu_0^{IV} &= u_1 \nu_1^I + 7u_1^2 \nu_1^{II} + 6u_1^3 \nu_1^{III} + u_1^4 \nu_1^{IV} \\ \nu_0^V &= u_1 \nu_1^I + 15u_1^2 \nu_1^{II} + 25u_1^3 \nu_1^{III} + 10u_1^4 \nu_1^{IV} + u_1^5 \nu_1^V \\ \nu_0^{VI} &= u_1 \nu_1^I + 31u_1^2 \nu_1^{II} + 90u_1^3 \nu_1^{III} + 65u_1^4 \nu_1^{IV} + 15u_1^5 \nu_1^V + u_1^6 \nu_1^{VI}, \end{aligned}$$

and so on.

From these it is evident that, knowing the series of differential coefficients of  $\nu$  with respect to  $u$  at any integral value such as  $\nu=5$ , the corresponding series may be obtained step by step down to  $\nu=0$ . In this way we obtain, for the series of coefficients

$$\frac{1}{2} \cdot \frac{1}{k!} \frac{d^k \nu}{du^k}$$

the values:

$k$ .	True Value.	Approximation.	Error.	Remainder.
1	·818,202,78	·833,333,33	+ ·015,130,55	- ·000,481,94
2	·916,762,37	·916,666,67	- ·000,095,70	- ·000,386,24
3	·944,923,44	·944,444,44	- ·000,479,00	+ ·000,092,76
4	·958,266,12	·958,333,33	+ ·000,067,21	+ ·000,025,55
5	·966,634,08	·966,666,67	+ ·000,032,59	- ·000,007,04
6	·972,225,35	·972,222,22	- ·000,003,13	- ·000,003,91

The table shows in parallel columns (i) the values derived from the reduction formula from those at  $\nu=5$ , (ii) the values given by the approximation  $1 - 1/6k$ , (iii) the differences between these values, (iv) the remainder

of the deviations needed to make up the total +014,648,61. The mere fact that this difference decreases at every step, and is finally reduced to a very trifling value, indicates that the errors shown in the first six terms, small as they are, are far greater than those to be anticipated at higher values of  $k$ .

The second functional equation, appropriate for variability maintained by a constant supply of mutations, has the form

$$\phi(e^{x-1}) - \phi(x) = 1 - x;$$

substituting here  $u_\nu = x$ , we have

$$\phi(u_{\nu+1}) - \phi(u_\nu) = 1 - u_\nu;$$

but from the recurrence formula

$$\frac{d}{d\nu} u_{\nu+1} = e^{u_\nu-1} \frac{d}{d\nu} u_\nu,$$

hence

$$\log \frac{d}{d\nu} u_{\nu+1} - \log \frac{d}{d\nu} u_\nu = u_\nu - 1;$$

so the functional equation may be written

$$\phi(u_{\nu+1}) + \log \frac{d}{d\nu} u_{\nu+1} = \phi(u_\nu) + \log \frac{d}{d\nu} u_\nu,$$

which is satisfied if

$$\phi(u) + \log \frac{du}{d\nu}$$

is a constant. Since by its definition  $\phi(0) = 0$ , we thus find that  $\phi$  is the same function of  $x$  as

$$\log \nu^f - \log \nu_0^f$$

is of  $u$ . The approximate form,

$$\nu = \frac{2}{1-u},$$

gives

$$\log \nu^f = \log 2 - 2 \log (1-u);$$

so that an approximation is given by

$$\phi(x) = -2 \log (1-x) = 2x + \frac{2}{2}x^2 + \frac{2}{3}x^3 + \dots,$$

which will account for the whole frequency save for

$$\log 2 - \log \nu_0^f = .200,645,07.$$

The frequency at  $p = k/2n$  is now found to be  $2/k$  when  $k$  is large, or the frequency element to be

$$\begin{aligned} df &= \frac{2dp}{p} = \frac{\sin \theta d\theta}{1 - \cos \theta} = \frac{(1 + \cos \theta)d\theta}{\sin \theta} \\ &= (\operatorname{cosec} \theta + \cot \theta)d\theta, \end{aligned}$$

thus confirming the solution obtained by means of the differential equation. By the present method, however, we can evaluate the total number of factors maintained in the specific variance by one mutation in each generation as

$$2(\gamma + \log 2n) + \cdot 200,645,07,$$

the value of which ranges from 30.372 to 57.903 as  $n$  changes from  $10^6$  to  $10^{12}$ .

The exact terminal frequencies for this case may be obtained from

$$v^I = v_0^I + uv_0^{II} + \frac{u^2}{2}v_0^{III} + \dots;$$

hence

$$\log \frac{v^I}{v_0^I} = \log \left\{ 1 + u \frac{v_0^{II}}{v_0^I} + \frac{u^2}{2} \frac{v_0^{III}}{v_0^I} + \dots \right\},$$

which, on expansion in powers of  $u$ , yields the frequency coefficients of the following table:

$k$ .	True Value.	Approximation.	Error.	Remainder.
1	2.240,917,26	2.000,000,00	- .240,917,26	+ .040,272,19
2	.953,776,16	1.000,000,00	+ .046,223,84	- .005,951,65
3	.671,863,62	.666,666,67	- .005,196,95	- .000,754,70
4	.501,095,71	.500,000,00	- .001,095,71	+ .000,341,01
5	.399,761,71	.400,000,00	+ .000,238,29	+ .000,102,72

showing, as in the previous case, that the discrepancy from the approximate formula is confined, for all practical purposes, to the extreme terminal values.

## 7. THE EFFECTS OF A SMALL SELECTIVE ADVANTAGE OR DISADVANTAGE.

The method of functional equations has now made clear in what way the terminal forms of the solutions of the differential equations should be interpreted; we may therefore now consider the differential equation appropriate to mutations enjoying a small selective advantage, such supplying in all probability the greater portion of the genetic changes taking place in the course of evolution.

If  $\alpha$  is the selective advantage of the mutant genes, the flux past any value of  $\theta$  may be written as

$$\frac{1}{2}\alpha y \sin \theta - \frac{y}{4n} \cot \theta - \frac{1}{4n} \frac{\partial y}{\partial \theta}, \quad \dots \quad (5)$$

provided  $\alpha^2$  may be neglected. It should be noted that the equation will only be correct if  $\alpha^2 n$  is a small quantity, and this limits its application

to very minute selective intensities. For these, however, the equilibrium condition of constant flux yields a differential equation for  $y$  of the first order, which may be written

$$y' - (2an \sin \theta - \cot \theta)y = -4anA$$

and may be integrated in the form

$$ye^{2an \cos \theta} \sin \theta = 2Ae^{2an \cos \theta} + B.$$

Since  $\cos \theta = -1$  when  $\theta = \pi$ , the condition that at this terminus, where no mutations are occurring,  $y \sin \theta$  should be zero, is that

$$B = -2Ae^{-2an},$$

giving the solution

$$y = 2A \operatorname{cosec} \theta (1 - e^{-2an(1 + \cos \theta)}).$$

At the terminus  $\theta = 0$  this will correspond to the distribution in equilibrium with one mutation per generation if

$$A = \frac{2}{1 - e^{-4an}},$$

so that the distribution adopted is

$$\begin{aligned} df = y d\theta &= 4 \operatorname{cosec} \theta \frac{1 - e^{-2an(1 + \cos \theta)}}{1 - e^{-4an}} d\theta \\ &= \frac{2}{1 - e^{-4an}} \left\{ 1 - e^{-4an/(1 + \epsilon^2)} \right\} dz. \end{aligned}$$

Fig. 1, C, shows the distribution on the scale of  $z$  for  $an = 1$ , while the curve A on the same figure shows the curve for factors at a minute selective disadvantage,  $an = -1$ .

While the curve of continuous distribution represents the frequencies well over that part of the range in which  $\pm z$  is considerably less than  $\log n$ , the termini of the distribution are subject to adjustments similar to those investigated in the absence of selection. Thus at the terminus  $\theta = 0$ , the frequency element  $4 \operatorname{cosec} \theta d\theta$  will be replaced by a series of frequencies for 1, 2, 3 genes given approximately by the series  $2, 1, \frac{2}{3}$ . . . , while at the terminus  $\theta = \pi$  we have the frequency element

$$\frac{8an \operatorname{cosec} \theta (1 + \cos \theta)}{1 - e^{-4an}} d\theta,$$

the limit of which is

$$\frac{4an \sin \theta d\theta}{1 - e^{-4an}}.$$

the form appropriate to steady extinction without mutation, the rate of extinction at this terminus being

$$\frac{2a}{1 - e^{-4an}}$$

in each generation; this rate may equally be obtained by substituting in the assumed flux of factors,  $Aa$ , the solution

$$A = \frac{2}{1 - e^{-4an}}.$$

The probability of a mutant, enjoying a small selective advantage  $a$ , spreading until it establishes itself throughout the entire population is thus found to be  $2a/(1 - e^{-4an})$ ; it is easy to see that with an indefinitely large population, or in any case if  $4an$  is large, this expression reduces to  $2a$ . Thus a mutation conferring a selective advantage of 1 per cent.

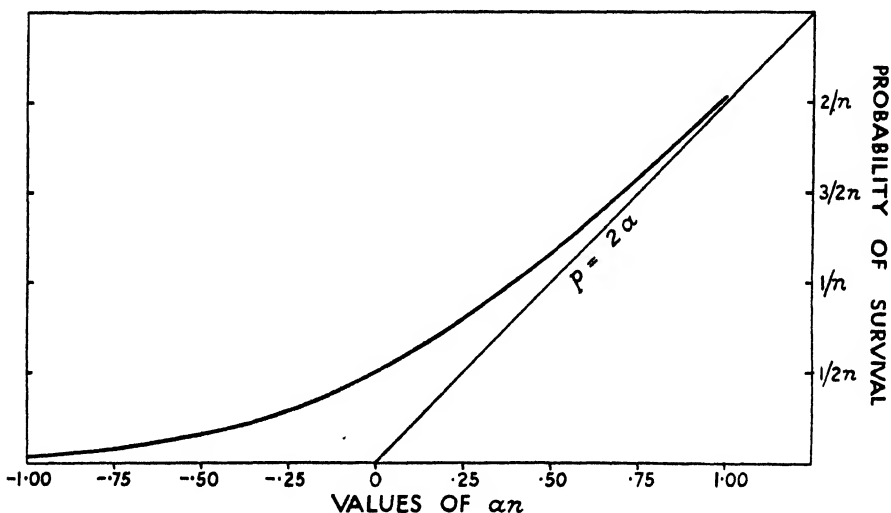


FIG. 2.—Probability of success for mutations having a very minute selective advantage or disadvantage.

will have practically a 2 per cent. chance of establishing itself. The value of this probability affords a means of checking the accuracy of our solution for values of  $a$  which, while still small, are large enough to vitiate the condition that  $a^2n$  should be small, the condition subject to which the differential equation has been obtained. For, in an indefinitely large population, the exact probability of ultimate survival is given by  $1 - U$ , where  $U$  satisfies the equation

$$U = e^{c(U-1)}$$

and  $c = e^a$ .

Writing  $P$  for  $1 - U$ , we have

$$cP = -\log(1 - P) = P + \frac{1}{2}P^2 + \frac{1}{3}P^3 + \dots$$

which is satisfied by

$$P = 2a - \frac{5}{3}a^2 + \frac{7}{9}a^3 - \frac{131}{540}a^4 \dots,$$

showing that when  $a$  is small, even though  $a^2n$  may be large, the value  $2a$  is a good approximation to the probability of survival.

When  $an$  is not large, the probability  $2a/(1 - e^{-4an})$  tends to the small but finite value  $1/2n$ , as  $a$  tends to zero, and is finite even for negative values of  $a$ ; its value changes, however, very rapidly as we pass from small negative to small positive selective advantages. Fig. 2 shows the course of this change. It will be observed that the probability of success increases over fiftyfold ( $e^4$ ) in passing from  $an = -1$  to  $an = +1$ , that is from distribution A to distribution C of fig. 1.

### 8. CONTRIBUTIONS TO THE VARIANCE.

In previous work the calculations of the quantitative contribution of different classes of factors to the total variance of the species has been much complicated by the widespread phenomenon of dominance, and by our ignorance of the conditions under which factors may be expected to be dominant or recessive. With the extension of genetical experience it now seems probable that the recessive character is characteristic of deleterious mutations which have long persisted in regular occurrence in the species or group in which they are known; and in the case of stable dimorphism, determined by a simple Mendelian factor, of the less favoured of the two phenotypes (genetic selection being necessarily absent or balanced in such cases). Consequently, it is probable that the new and sometimes favourable mutations on which evolutionary progress must rely are neither dominant nor recessive, but have heterozygotes of an intermediate character. Their contribution to the variance will then be simply proportional to  $pq$  or to  $\sin^2 \theta$ , and the total variance supplied by mutations having a selective advantage  $a$ , for each one occurring per generation, will be proportional to

$$\int_0^\pi \frac{1 - e^{-2an(1 - \cos \theta)}}{1 - e^{-4an}} \sin \theta d\theta$$

or to

$$\frac{2}{1 - e^{-4na}} - \frac{1}{2an}.$$

For negative values of  $an$  exceeding 2 this is nearly equal to  $1/2an$ , while for large positive values it approaches a constant value of 2, passing through the value unity when  $a=0$ . Its course is shown in fig. 3. If in the immediate neighbourhood of neutrality beneficial and harmful mutations are equally frequent, the variance contributed by mutations in a given range of utility will increase sharply as the utility is increased past the

point of neutrality. For higher values of  $a$  there is every reason to suppose that the supply of mutations falls off, so that there will be a maximum in the contributions to the specific variance ascribable to slightly beneficial mutations. The frequency of harmful mutations probably increases considerably with the extent of the injury up to high values of  $-an$ ; in spite of the decrease in the average contribution of each mutation to the specific

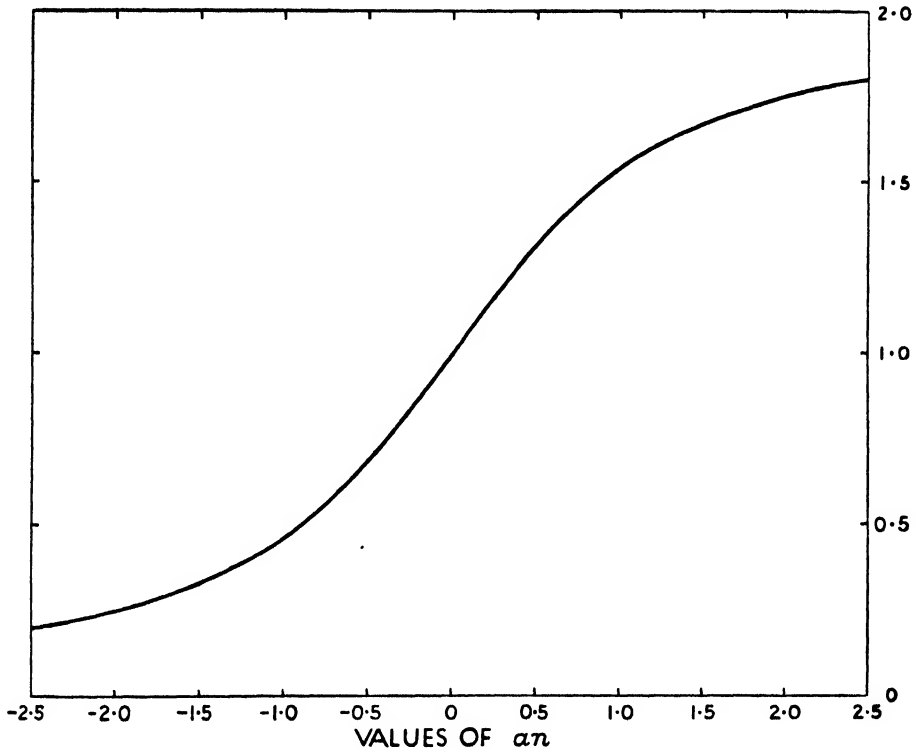


FIG. 3.—Proportionate contribution to the specific variance for factors of varying selective advantage.

variance, there may thus well be a second maximum, representing the contribution of definitely deleterious mutations which are constantly kept rare by counter-selection. This latter maximum is of no direct importance for evolutionary change, though the effects of Natural Selection in reducing persistent mutants of this class to the recessive condition seem to be of the greatest interest. The portion of the genetic variance to which evolutionary progress is to be ascribed may be a large or a small portion of the whole observable variance, but seems in any case to be concentrated in groups of factors each determining a very minute selective advantage.

## 9. SUMMARY.

The discussion of the distribution of the gene ratio of the author's paper of 1922 is amended by the use of a more exact form of the differential equation to be satisfied. It appears that the time needed to halve the variance by random extinction of genes in the total absence of mutations should be 1.4 instead of 2.8 times the number of potential parents in each generation. Either value shows that the loss of variance due to this cause is too trifling to be appreciable in the balance of causes which maintain the actual genetic variability of species.

The same correction alters the distribution appropriate for the maintenance of variability at a fixed level by mutations in the absence of selection. The new solution closely resembles the form previously obtained and now confirmed for the practical case in which selection is present. The method of differential equations, however, fails to deal satisfactorily with these cases, owing to the failure of the integrals to converge at the termini representing cases in which one or other allelomorph is extremely rare.

A method of functional equations is developed for dealing with the termini, and is shown to lead to the same solutions as the amended differential equations in the central portion of the range for which the latter are valid, and further to give the terminal distribution of rare allelomorphs. The method requires the investigation of a continuous function  $u_v$  of argument  $v$  satisfying the recurrence formula

$$u_{v+1} = e^{u_v - 1}.$$

From the asymptotic form of this function its expansion in the neighbourhood of  $u=0$  is derived, giving the frequencies of the required distributions.

Exceedingly minute values for the selective advantage or disadvantage make a great difference to (i) the chance of success of a mutation and (ii) the contributions of such mutations to the specific variance. The order of magnitude to be considered is the inverse of the population of the species. The neutral zone of selective advantage in the neighbourhood of zero is thus so narrow that changes in the environment, and in the genetic constitution of species, must cause this zone to be crossed and perhaps recrossed relatively rapidly in the course of evolutionary change, so that many possible gene substitutions may have a fluctuating history of advance and regression before the final balance of selective advantage is determined.



## THE EVOLUTION OF DOMINANCE IN CERTAIN POLYMORPHIC SPECIES

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### 1. SELECTIVE EQUILIBRIUM IN POLYMORPHIC SPECIES

IN his very interesting contribution to the problem of the evolution of dominance, published in the AMERICAN NATURALIST of January, 1930, Mr. J. B. S. Haldane calls attention to the peculiar dominance phenomena found by Nabours in the grouse locusts *Paratettix* and *Apotettix*, and by Winge in the fish *Lebistes reticulatus*. These, and I shall suggest that some of the land-snails such as *Helix hortensis* and *H. nemoralis* should be added to the list, have each of them three remarkable characteristics in common. The wild forms are all visibly polymorphic. The polymorphism may in each case be shown to be determined by genes or gene-complexes which are very closely linked in inheritance. Finally, each species has a relatively common "universal recessive," together with a number of usually less common dominant forms, which, if allelomorphic, show no mutual dominance, but have heterozygotes combining the characteristics of the two dominant homozygotes. The recessiveness of the "universal recessive," apart from rare deviations in some compound types produced in culture, seems to be complete.

Haldane had previously suggested with respect to the grouse locusts, and Demerec later with respect to *Lebis-*

tes, that the close linkage and frequent apparent allelomorphism observed in these groups was due not only to the infrequency of crossing over in their chromosomes, but to several chromosomes received from the same parent being generally transmitted in a group to the same offspring. Haldane now, with the support of C. D. Darlington and C. L. Huskins, has suggested that such linkage between chromosomes may be accounted for by sectional translocations, and that the dominant genotypes are themselves due to the duplication of such translocated segments. While some explanation of this kind appears to the writer extremely attractive in respect of the very close and frequent linkage observed, it is not so plain in what manner Haldane finds in it an explanation of the dominance phenomena. He says, "On the theory here adopted we need only suppose that some of the genes in the duplication have a greater effect when three or four are present than when only two are found, as in the normal type." This would indeed suffice to explain why the heterozygous duplication should differ from the universal recessive. But it gives no explanation of the completeness of dominance; it does not explain why the homozygous duplication should resemble the heterozygote. For this it is necessary to suppose, not only that some of the genes in the duplication have a greater effect when three are present, but also that none of them should exert a further effect when the three are increased to four; and this curious type of limitation must be observed in each of the possible duplications to which the different dominants are to be ascribed. Attractive as the suggestion is, therefore, in respect to linkage, it can not be regarded, as it stands, in any sense as an explanation of the dominance observed in these groups.

In previous papers (Fisher, 1928 *a* and *b*) I have developed a theory of the evolution of dominance by the natural selection of modifying factors, and have applied it to explain the generally observed recessiveness of deleterious mutants exposed to counter-selection, yet maintained at an extremely small frequency in the stock

by persistent mutation. Haldane seems to draw an analogy between such mutants and the less numerous genotypes of polymorphic species without sufficient circumspection. There is no good ground for regarding the less numerous genotypes of polymorphic species as subjected to counter-selection and only maintained in the stock by persistent mutation; indeed, the frequency with which they appear in nature would, unless the counter-selection postulated were exceedingly minute, require such enormous mutation rates as would inevitably have been detected in much less extensive experiments than those of Nabours and Winge. Mendelizing polymorphism may, on the contrary, be maintained indefinitely, as is probably the case with several polymorphic butterflies (Fisher, 1927), by a balance of selective actions, under which the frequency ratio of the contrasted genes settles down to a condition of equilibrium, which will be stable, to take the simplest case, if the heterozygote is at a selective advantage compared to both homozygotes. Since in the opposite case, when the heterozygote is the least advantageous phase, the equilibrium will be unstable and one or other homozygote must be exterminated, we should not expect to find such factors in nature. Stability, brought about by a favored heterozygote, may, on the contrary, be not uncommon, and there is some evidence in the case of the butterflies alluded to that the opposing selective forces consist on the one hand of the bionomic advantage of a mimetic or otherwise advantageous coloring, and on the other of some physiological factor such as inferior viability or fertility, associated with the dominant homozygote. Such cases appear to offer the unparalleled opportunity of estimating the magnitude of a bionomic advantage in nature by direct observation of the frequency of contrasted genotypes in the wild population, together with the experimental determination of the extent of inviability or sterility.

In this regard Nabour's genetical study of the grouse locusts has opened out a field of extraordinary interest. Haldane's remark, "Now on Fisher's theory there is no

obvious reason why modifiers should not be able to suppress the effect of a duplication as easily as that of a gene," may now be accepted in its full force, for there is no reason to suppose that the effects of the duplications, if duplications they are, have been exposed to counter-selection. Indeed, there are two reasons why the selection of dominance modifiers should be particularly effective in such cases. In the first place, the heterozygotes in which the effect of such modifiers is exposed to selection are now a perceptible percentage of the wild population, instead of having a frequency of the order of 1 in 10,000. This must increase the selective intensities about 100 or 1,000 fold, so that the difficulty felt by critics such as Wright and Haldane, who feel that in the case of deleterious mutations the selective intensities at work are too minute to have produced great results, is here removed. In the second place, if, as Haldane suggests, the dominant forms are due to a duplicated tract of chromatin, the evolutionary modification of this tract will take place almost wholly in heterozygous individuals. The selective advantage of any gene-contrasts within this tract will be totally unaffected by any reaction they might have produced in the "universal recessive," or in other dominant types. One may say that such a portion of chromatin is reserved for the evolutionary improvement of each particular heterozygote, and in less degree of the corresponding dominant homozygote.

Even without postulating duplication, a similar result will follow if, as is made probable by the high linkages observed, each dominant gene is situated in a section of chromatin in which crossing over never takes place. The evolutionary progress of such a section would then be wholly conditioned by the quality of its effects in the presence of the dominant gene. The modification of the extent of dominance through evolutionary changes in permanently associated genes bears some resemblance to Haldane's own views in depending on the selection rather of multiple allelomorphs than of multiple factors, for he has suggested that multiple allelomorphism is also re-

sponsible for the evolution of dominance against single defective gene mutations—an important suggestion, though made, I believe, for the wrong reason, for it is difficult to believe that the evolution will be hastened by throwing the whole burden on to one factor only out of the many thousands present, even though this one is clearly the most relevant.

The severest test of a theory is to build upon it a system of inferences, for if any rigorously logical inference is found to be untrue the theory fails. If, on the contrary, facts previously unsuspected are inferred from the theory, and found on trial to be true, the theory is undoubtedly strengthened. The plain inference from the theory of the modificatory selection of dominance and the fact that one particular pattern in the grouse locusts is recessive to all its natural alternatives is that this particular pattern is less advantageous than any of the others. This may seem surprising since this particular pattern is often, perhaps always, the most numerous in nature. We have, however, inferred only one half of the selective balance. The selective advantage of the heterozygote over the homozygous recessive is inferred from the fact of dominance to be due to its pattern. If there is a balance of selective agencies, the heterozygote must also enjoy some advantage over the homozygous dominant. In this contrast the patterns are alike, and we should naturally look for some constitutional difference affecting viability or fertility. Such a constitutional deficiency again accords well with the theory that we have to do with a homozygous duplication. It is of more direct consequence to our argument that a deficiency of homozygous dominants of sufficient magnitude to be statistically significant appears from the published record of Nabour's breeding experiments.

## 2. VIABILITY OF HOMOZYGOTES

In order to examine the evidence as to the viability of homozygotes, an exhaustive examination was made of the

very large body of data reported by Nabours in his experiments with *Apotettix* (Nabours, 1925).

Matings from which homozygotes of the dominant forms appear are necessarily unsuitable for studies of linkage. Only a minority of Nabour's experiments can therefore be available for studying their viability. In matings of the generalized type  $P/Q \times P/Q$  homozygotes and heterozygotes should appear in equal numbers, or, strictly, in proportion to their viability up to the time of classification. A comparison between the observed frequencies in such matings should, therefore, if the material proves to be sufficiently ample, supply an indication of any existing difference in viability. These matings will, however, show a certain small proportion of recombined types, some of which are not phenotypically separable from the three main types of offspring, and although the frequency of recombination is small, it is important that the treatment of the recombined types should allow of no bias in the massed comparisons.

Of the four recombined types which should appear in equal numbers two, namely,  $P/+$  and  $Q/+$ , will have been classified as homozygotes, while the remaining two,  $P/PQ$  and  $Q/PQ$ , will generally have been classed as heterozygotes; exceptionally, as with the mating  $Y/T \times Y/T$ , these latter classes are recorded separately, and in such cases they have been added to the heterozygotes. If viability is equal, crossing over thus introduces no bias into the main comparison, though by classifying a small proportion of homozygotes as heterozygotes and an equal proportion of heterozygotes as homozygotes the effect sought for will certainly be diluted.

If  $p$  is the recombination fraction, and  $q = 1 - p$ , the viability ( $v$ ) as determined from the frequency ratio of the offspring as classified will be related to the true viability  $v_0$ , by the equations

$$v = \frac{qv_0 + p}{q + pv_0},$$

$$v_0 = \frac{qv - p}{q - pv}.$$

which may be used to correct the estimates obtained. Such corrections can not, of course, influence the test of significance.

Many of the matings are, however, of the type  $PQ/R \times PQ/R$ . In addition to the possible recombination of  $PQ$  and  $R$ , we have here also the two additional types of recombination involving the separation of  $P$  from  $Q$ . Thus the recombined gametes  $P$  and  $QR$  will yield zygotes  $PQ/P$  and  $PQ/QR$  which will be classified as  $PQ/PQ$  and  $PQ/R$ , respectively, together with two independently classified zygotes  $R/P$  and  $R/QR$ . Bias may in this case be avoided by classing these with  $R/PQ$  and  $R/R$ , respectively, and the same convention is applied in the classification of the recombined zygotes  $R/Q$  and  $R/PR$ , whenever they occur. In this case also there will be some dilution of the effect sought for, but its test of significance is unaffected.

Table I gives the frequencies for the forty types of mating available, the reference numbers in the first column giving the first mating of each type. The crossovers capable of separate classification are in each case shown on a separate line, in the column to which they have been assigned. In the aggregate 4,309 homozygotes and 4,617 heterozygotes survived to be classified. This is a very considerable defect of homozygotes, not to be ascribed to chance, since it exceeds three times its standard error. The massed data demonstrate an appreciable defect of homozygotes. The percentage viability is about 92.9 per cent. of that of the heterozygotes.

In respect of individual dominants the data are necessarily less ample, though here also there are some cases which must be judged to be individually significant, and in all these the deficient class is that of the homozygotes. Table II shows the aggregate of homozygotes and heterozygotes for each dominant or compound of dominants tested. The dominants  $Y$ ,  $O$  and  $R$  and the compounds  $RK$ ,  $YK$  and  $MR$  all show individually significant deficiencies, while  $M$ ,  $T$ ,  $G$ ,  $Z$ ,  $YT$  and  $YZ$  all show deficiency in less degree. The cases of  $RK$  and  $YK$  are especially

TABLE I  
MATINGS OF TYPE P/Q × P/Q

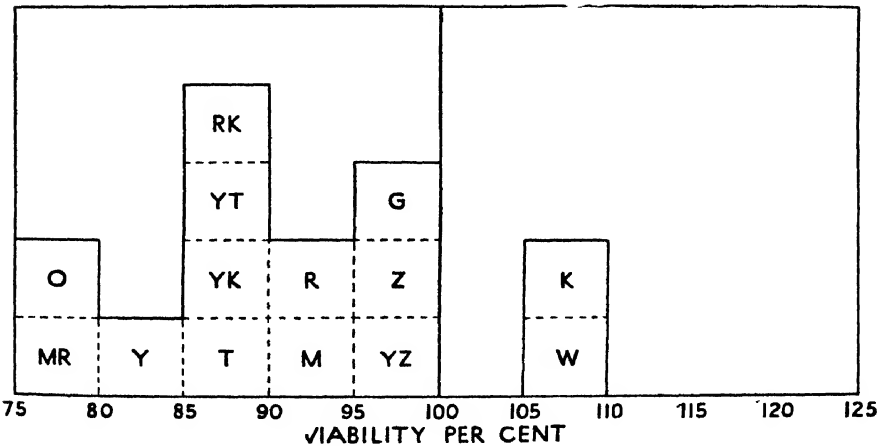
Mating	Parents	Homo- zygotes	Hetero- zygotes	Homo- zygotes	Crossovers
370	H/G	15	28	15	
375	M/R	49	115	54	
391	M/W	27	47	26	
397	M/T	58	95	54	
399	M/G	76	147	83	
405	M/K	238	556	292	
422	Y/O	9	26	9	
431	Y/R	65	185	86	
439	Y/Z	71	145	72	
447	Y/T	12	48	22	
452	YT/O	14	42	14	
458	Y/K	33	69	37	
470	O/R	16	46	30	
471	O/T	18	24	14	
474	O/G	40	105	51	
482	O/K	8	28	8	
487	R/T	35	90	32	
492	R/K	88	168	100	
510	W/K	74	138	75	
517	Z/K	20	35	13	
524	Z/T	25	35	15	
525	T/G	32	62	27	
528	T/K	40	64	35	
541	MR/K	69	181	110	5
			2	3	
553	M/RK	85	167	73	6
569	YZ/T	49	85	25	
			5	1	8
571	YZ/G	138	258	110	
			4	4	13
577	YZ/K	96	192	103	
			8	5	1
584	YT/R	134	312	149	
			—	1	8
605	YK/O	21	48	22	
612	YK/R	184	423	158	8
			4	4	
623	YK/Z	18	43	9	8
624	YK/T	51	108	48	
			7	1	9
627	YT/K	29	35	20	
			6	3	1
631	RK/T	142	315	138	
			—	1	3
635	B/MRK	—	1	—	
636	MRK/T	5	20	4	3
640	YK/RK	—	9	4	
641	YRK/G	8	7	5	3
			2	1	
643	YZ/RK	27	77	24	
Total	.....	2,119	4,617	2,190	



TABLE II

Factor	Homozygotes	Heterozygotes	Estimated viability per cent.
Dominants showing significant deficiency			
Y .....	190	473	80.3
O .....	127	319	79.6
R .....	605	1343	90.1
RK .....	243	568	85.6
YK .....	274	642	85.4
MR .....	69	183	75.4
Dominants showing non-significant deficiency			
M .....	533	1127	94.6
T .....	427	958	89.1
G .....	296	613	96.6
Z .....	126	258	97.6
B .....	0	1	—
YT .....	177	395	89.6
YZ .....	310	629	98.6
MRK .....	5	21	—
Dominants showing non-significant excess			
K .....	804	1482	108.5
W .....	100	185	108.1
H .....	15	28	—
YRK .....	8	9	—
Total	4309	9234	93.3

interesting, since K is the only dominant for which substantial data are available which by itself shows no deficiency of homozygotes. The only other cases of excess



are shown by W with only 285 observations, by H with only 43 and by YRK with only 17.

Fig. 1 shows the distribution of the fourteen viabilities, based each on over 200 survivors. The true viabilities will, as has been seen, be generally somewhat lower than those shown, which have not been corrected for dilution due to recombination.

The deliberate experimental determination of the selective viabilities of different homozygous and heterozygous types would evidently be of great value in clearing up the situation. In particular, though, on the view suggested, there is no reason to anticipate that the viability of the homozygous recessives should be inferior to that of the heterozygotes, there is some, though still inconclusive, evidence from Nabour's matings that this homozygote also may be at a slight disadvantage. Four types of matings are available on this point.

(i) When homozygous recessives (+/+) are mated with heterozygotes (+/Q) where Q stands for a dominant or compound of dominants, homozygotes and heterozygotes are expected in equal numbers. In this case all cross-overs in the dominant compound are recognizable, and may be omitted. These matings are shown in Table III.

TABLE III. +/×+/Q

Mating	+/+	+/Q .
4	20	8
5	3	6
6	33	32
351	6	9
721	31	36
Total	93	91

This group gives 93 homozygous recessives to 91 heterozygotes, and shows no defect of homozygotes.

(ii) When like heterozygotes (+/Q) are interbred, a quarter homozygous recessives are expected, but the homozygous dominants can not be distinguished from the heterozygotes; only two of the four crossover classes are

distinguishable, the other two being confounded with the heterozygote and homozygous dominants. We may therefore subtract the number distinguishable from the number in this class. These matings are shown in Table IV.

TABLE IV.  $+/Q \times +/Q$ 

Mating	$+/+$	Q	Crossovers
287	77	114	—
292	2	10	—
312	4	12	—
316	5	24	—
348	5	16	—
352	28	82	—
365	24	81	—
443	14	52	4
469	32	118	11
521	26	91	—
1246	4	11	1
	221	611	16
		16	
		595	

This group gives 221 recessives to 595 heterozygous and homozygous dominants, and shows an appreciable excess of recessives. This is due, however, principally to the great excess shown in mating 287, which the other matings as a whole tend partially to counterbalance.

(iii) When heterozygotes  $+/P$ ,  $+/Q$  are mated, the expectation is again a quarter recessives. If P and Q are compounds containing no dominant in common all the non-recessives will be heterozygotes, and all crossovers will be theoretically capable of detection. Those detected have therefore been omitted; Table V shows eighty-six types of mating of this class.

These give in all 1,732 recessives to 5,466 heterozygotes, a deficiency of recessives which only just falls short of twice the standard error. It is the material in Table V which makes it worth while to call attention to the possibility of making more direct tests of the viability of the recessive type, for though the data as they stand can not

TABLE V.  $+/P \times +/Q$ 

Mating	$+/+$	Hetero- zygous	Mating	$+/+$	Hetero- zygous	Mating	$+/+$	Hetero- zygous
275 .....	5	21	755 .....	38	118	1085 .....	11	55
277 .....	22	103	757 .....	36	142	1099 .....	27	55
282 .....	15	63	837 .....	2	6	1103 .....	1	1
283 .....	11	54	842 .....	16	93	1104 .....	1	13
285 .....	43	102	843 .....	104	332	1106 .....	8	33
291 .....	13	38	858 .....	29	83	1113 .....	28	87
293 .....	13	46	860 .....	0	8	1114 .....	44	143
294 .....	5	27	866 .....	6	26	1115 .....	34	75
295 .....	26	73	868 .....	10	21	1118 .....	41	102
303 .....	36	119	889 .....	15	38	1160 .....	50	159
311 .....	24	62	890 .....	12	25	1163 .....	22	67
314 .....	8	24	901 .....	7	27	1164 .....	90	241
315 .....	0	5	990 .....	0	1	1210 .....	0	29
324 .....	1	11	1011 .....	13	74	1221 .....	45	121
333 .....	5	19	1013 .....	8	37	1234 .....	15	53
338 .....	11	42	1015 .....	3	11	1236 .....	6	34
341 .....	13	33	1017 .....	10	29	1247 .....	12	37
346 .....	68	234	1030 .....	2	14	1248 .....	25	39
349 .....	16	41	1031 .....	35	155	1270 .....	5	23
353 .....	5	17	1032 .....	14	58	1273 .....	22	58
354 .....	24	64	1033 .....	25	62	1281 .....	13	50
647 .....	25	84	1054 .....	56	157	1282 .....	29	105
649 .....	40	141	1055 .....	1	2	1285 .....	0	2
653 .....	25	71	1056 .....	27	61	1290 .....	17	41
674 .....	2	15	1066 .....	17	75	1292 .....	88	264
691 .....	5	8	1068 .....	1	10	1376 .....	51	162
709 .....	12	37	1072 .....	7	15			
711 .....	3	5	1082 .....	53	114			
734 .....	1	3	1083 .....	4	2			
748 .....	16	56	1084 .....	3	3			
Subtotal ...	493	1618	Subtotal ...	554	1799	Subtotal	685	2049
						Total	1732	5466

be judged as showing a significant deficiency of recessives, they do raise the question whether there is not in reality such a deficiency, either of slight extent, or even possibly as great as has been found for some of the homozygote dominants.

(iv) A group of matings of type  $+/PR \times +/QR$  produce not only a recognizable quarter of recessives, but also homozygotes in R distinguishable from the heterozygotes. In respect of crossing over between Q and R, two of the crossover classes will appear among the heterozygotes and dominant homozygotes respectively, while

the two other classes are recognizable. We therefore subtract half the observed crossovers from each of these two classes; other types of crossovers are recognized and may be ignored. Table VI gives the matings of this group.

TABLE VI  
MATINGS OF TYPE + /PR × + /QR

Mating	+ / +	Heterozygous	Homozygous	Crossovers
366	14	25	11	—
390	11	35	13	—
1069	3	7	2	—
1117	68	146	71	—
1119	21	61	25	6
	117	274	122	6
		3	3	
		—	—	
		271	119	

In this group the recessive homozygotes show a deficiency even greater than that shown by the dominant homozygotes; the data, are, however, quite insufficient to stand alone. They may, by throwing heterozygous and homozygous dominants together, be combined with Table IV showing together just such a slight excess of reces-

Table IV	221	595
Table VI	117	390
	—	—
	338	985

sives as might be ascribable to the lower viability of the homozygous dominants, though the ratios are too heterogeneous for such an interpretation to be relied on. Finally we may throw together all the evidence on homozygous recessives by calculating from each table the expectation, based where possible on the heterozygotes observed.

The deficiency of eighty-four in all is, of course, not significant, though it may well be that the homozygous recessives are really less viable than the heterozygotes to the extent of about 3 or 4 per cent.

TABLE VII  
SUMMARY FOR RECESSIVES

	Expected	Observed
Table III . . . . .	91	93
Table IV . . . . .	198	221
Table V . . . . .	1,822	1,732
Table VI . . . . .	136	117
Total . . . . .	2,247	2,163

The finding of inferior viability among the homozygous dominants in *Apotettix* supports the view that the dominant patterns are in nature advantageous, not only by verifying a previously unsuspected fact deduced from this view, but by opening out the possibility of the direct experimental determination of the magnitude of the selective advantage of one color pattern over another in nature. For if  $a$ ,  $b$  and  $c$  are proportional to the numbers of offspring left, on the average, by homozygous recessives, heterozygotes and homozygous dominants, respectively, then the ratio of recessive to dominant gametes will tend to approach the equilibrium value  $(b - c)/(b - a)$ . If therefore we are given the frequency of the different types in the natural population and can also determine experimentally the relative viabilities, and if necessary, also the fertilities in culture, we shall be in a position to infer the existence, and if our data are sufficiently accurate, to calculate the magnitude, of any selective advantage not due to constitutional causes, which in nature favors the dominants over the recessive. With increasingly precise data upon these points therefore it should be possible to put this interpretation of the dominance phenomena in the grouse locusts to a quantitative test.

### 3. LEBISTES

The condition found by Winge (1927) in *Lebistes reticulatus*, while showing some striking similarities, also differs in two important respects from that found in the grouse locusts. These are, first, that, with one excep-

tion, the genes found are sex-linked in inheritance; and secondly, that, with the exception of the autosomal gene in its homozygous condition, they are without visible effects in the female (save in certain females of intersexual character). In this fish the male is the heterogametic sex, so that, without the transference of genes by crossing over from the X to the Y chromosome, or *vice versa*, it would be impossible to observe the phenomena of dominance in the sex-linked factors. Such transference has, however, been observed in several cases and, when the same factor is introduced into a male, both in its X and its Y chromosome, the fish is somatically like one into which it is introduced from one side or the other only. As far as is known, therefore, the sex-linked genes for color variants in the male may be said to be dominant, although it should be observed that Haldane's statement that "Winge found eighteen genes dominant to the normal and none recessive" is an induction from the cases so far observed, rather than a directly observed fact. The autosomal gene is also dominant in the male though it should be more properly described as recessive in the female, since it is only in the homozygous female that the striping (*zebrinus*) has been yet observed.

Dr. Winge informs me that the males are certainly polymorphic in nature, though the types obtainable from dealers are often more "beautiful" than those which can be found wild, thus suggesting that appreciable modification and gene combination have been brought about by human selection. His observations suggest the important conclusion that the X chromosome is usually "empty" (*i.e.*, the universal recessive), in wild specimens, and that the genes for additional color patterns in the males are, in nature, more fully confined to the Y chromosome than is the case in the domesticated breeds.

We have here a group of facts extremely suggestive of the interpretation, which we should at once infer from the view that dominance has been determined by selective agencies, namely, that the color variants are advantageous in the male and disadvantageous in the female.

Given the fundamental conditions for the stability of gene ratio, upon which any permanent Mendelizing polymorphism must rest, such a situation will lead, on our view, to consequences remarkably consonant with the facts observed. First, the variant form should become dominant in the male and recessive in the female fish; next, continued counter-selection in the female should obliterate entirely, in this sex, the effects of those genes which were capable of leaking into the X chromosome; thirdly, favorable selection in the males with counter-selection in the females should make the variants rarer in nature in the X chromosome than in the Y, although, in the absence of selection, crossing over should tend to equalize the proportions; fourthly, favorable selection in the Y chromosome, with counter-selection in the X, must constantly favor those genotypes in which linkage with the sex-determining portion of the Y chromosome is closest, and may thus have built up the system of close sex-linkage which is now found; fifthly, close linkage with Y may have enabled certain variants, beneficial in the male, to have established genetic stability, which, had they been autosomal, would have been definitely deleterious, and have never contributed to the natural polymorphism. It may be a coincidence that the one variant whose effect has not been entirely suppressed in the female is the only one that still stands outside the sex-linked system; even without unduly stressing this fact, however, it is difficult to imagine how the observed facts in *Lebistes* could more closely simulate those to be anticipated on the theory of the selective modification of dominance.

#### 4. *HELIX*

The facts so far available as to polymorphism in the land snails, for which *Helix hortensis* and *nemoralis* may be taken as typical, seem closely to resemble those in the grouse locusts. A great variety of distinct forms exists in nature, and of these one of very general occurrence, and frequently the commonest, appears to be a universal



recessive. Captain Diver (1929) has ascertained that various forms now found appear without perceptible change in the proportions among Pleistocene remains, and we may somewhat confidently infer from this a definite mechanism of stability in the gene-ratios. Breeding experiments, of which few unfortunately have yet been published, appear to show, so far, complete linkage between the different factors, although types which may have arisen originally by recombination are found in nature.

Thus we find the three peculiar features, of polymorphism, close linkage and the universal recessive type of dominance, occurring together in groups as widely separated as are mollusks, vertebrates and insects, and we can scarcely doubt that the three phenomena are associated causally. While it is at present certainly premature to choose one of these three as the primary cause responsible for the other two, it is perhaps worth while, even now, to consider, in a spirit of conjecture, whether the close linkage may not, at least in the snails and grouse locusts, have played the primary rôle.

##### 5. EVOLUTIONARY CONDITIONS INDUCED BY CLOSE LINKAGE

Close linkage within and especially between chromosomes introduces one difficulty to normal evolutionary development which has not, I think, been recognized. While its ultimate discussion will undoubtedly require a far more refined mathematical development than I can here attempt, it does seem possible to gain a crude qualitative notion of its nature by considering the extreme, and perhaps unreal, case of a species in which no recombination whatever is possible. Any considerable change, in the evolution of a species from its ancestral form at a remote geological period, must have involved numerous genetic substitutions. The genetic novelties ultimately adopted must, as far as we know, have originated in mutations, though where free recombination is possible, we need by no means confine ourselves to the supposition that such mutations must necessarily have been bene-

ficial from their first appearance. It is indeed certain that many species contain a large amount of latent variability by the selection of which their instantaneous rates of evolutionary improvement are maintained. There is no need, however, to suppose that the whole of this is due to a stream of mutations beneficial from their first appearance, in process of spreading over the species, rather than that much of it may be due to effectively neutral mutations which have occurred in the past, and the ultimate fate of which is at present in process of decision. However this may be, in a species entirely devoid of recombination, and possessing for genetic purposes only a single gene, a beneficial substitution which could be adopted without hindrance, in a species enjoying the advantage of free recombination, will be threatened by every other genetic substitution possible within the genetic variance of the species, and will inevitably be pushed aside and suppressed if any such alternative substitution offers a greater selective advantage. The different genotypes will, in fact, compete with one another like a system of multiple allelomorphs, and instead of genetic improvement being possible simultaneously in hundreds of different loci, the steps of evolutionary improvement will have to take place one at a time, the weaker always making way for the stronger.

The chance of any particular substitution being adopted may be crudely represented as the chance that during a certain time, during which it is spreading through the species, no substitution more suitable than itself makes its appearance. If we represent by  $a$  the selective advantage of a substitution and by  $\mu_a da$  the frequency with which substitutions in the range  $da$  present themselves, the time needed for the substitution to spread, within any assigned limits, over the population will be inversely proportional to  $a$ , while the frequency with which substitutions superior to any given level  $b$  appear will be represented by

$$\int_b^{\infty} \mu_a da$$

where  $\mu_a$  doubtless falls off rapidly as  $a$  is increased. Consequently if  $b$  represents a level of advantage which has a tolerable chance of survival, before some bigger advantage can thrust it aside, we shall have some such relation as

$$bc = \int_b^{\infty} \mu_a da$$

where  $c$  is a constant, dependent from the chance of survival chosen. For a suitably chosen constant,  $b$  may be regarded as a standard level of selective advantage such that substitutions giving a selective advantage greater than  $b$  can and do prevail over their competitors, and so contribute to evolutionary progress, while those conferring a much smaller selective advantage have scarcely any chance of doing so. There will, in fact, in such cases be something like a real *limen* of selective advantage, such as Sewall Wright has suggested even for cases involving free recombination. It is not unreasonable to suspect that the chance of success for selective advantages less than  $b$  may be proportional to  $e^{-b/a}$ , an expression that diminishes with extreme rapidity as  $a$  is diminished, although when  $a$  is so small that the expression approaches  $1/(2n)$ , where  $n$  is the number of parents breeding in each generation, the probability must remain nearly constant until  $a$  falls to zero.

For the more practical case, in which some degree of recombination is possible, the state of affairs appears at first sight to be similar, though with a larger value of the constant  $c$ , which may be expected to increase with increasing recombination, in proportion to the number of separable loci in the species; and, unsatisfactory as such a crude approach must be felt to be, it does seem to show with sufficient clarity that when linkage is, as with the species here considered, extremely close, it will be impossible to utilize a number of the smaller genetic improve-

ments which present themselves, because the whole of the existing nuclear material is fully occupied with matters of more importance.

Now the system of obtaining improved color patterns, such as appears to fit the facts with the grouse locusts, by dominant duplications, which are deleterious in the homozygous phase, at once raises the problem of why the universal recessive can not itself be modified to a more advantageous pattern, and so supersede the dominants with which it appears to be now in equilibrium. There is little harm in putting forward a conjecture so long as it is clearly recognized as such, and it appears to the writer possible that the color pattern of the grouse locust is not among the more important matters with which its evolutionary progress is urgently concerned. What these more important matters are it would be idle to guess—perhaps its digestion or its vision or its reproductive instincts may be of greater real importance to the insect; at least we can not fairly assume that the real importance of a feature is in any way measured by its conspicuousness to ourselves, and we have no reason at present to assume without question that the universal recessive, in species with such close linkage as the grouse locusts, is free to seize upon any such relatively trifling advantage as might be gained by modifying its color pattern.

This point of view brings out, I think, one of the most attractive features of the theory of duplications, for if modifications in color pattern are too unimportant to win their way to success in the germ-plasm of the universal recessive, it is clear that modifications of the heterozygote must be due to factors located elsewhere, and the theory supplies a tract of chromatin where such factors, though they may compete among themselves, are protected from outside competition. On this view the evolution of dominance would have taken place by the selective modification of the duplication itself. The same of course could be said, without duplication, of any tract permanently associated with the dominant gene.

## 6. SUMMARY

Polymorphism in wild populations must usually imply a balance of selective agencies, of which the simplest type is a selective advantage of the heterozygote over both homozygotes. Such a condition should not be confused with the maintenance of a rare mutant type against counter-selection by means of repeated mutations. While such mutations should on the theory of the selective modification of dominance tend to become recessive, heterozygotes in polymorphic species will tend to resemble in external appearance whichever homozygote it is most advantageous to resemble. The selective balance must then be maintained by some constitutional disadvantage of the homozygous dominant.

The modification of dominance should in such cases be especially rapid; partly by reason of the far greater frequencies of the heterozygotes exposed to selection, and partly, if any tract of chromatin is permanently associated with the dominant gene, from the fact that the evolutionary modification of such a tract will be reserved for the improvement of the heterozygote, and in less degree of the corresponding homozygote.

Nabours' experiments with the grouse locust *Apotettix* do, in fact, show such a deficiency of homozygous dominants as is required by this theory. The average amount of the deficiency is about 7 per cent. In six individual cases the deficiency is statistically significant, and six more show a non-significant deficiency, against two showing a non-significant excess.

It is not certain from the existing data whether the homozygous recessive is equivalent in viability to the heterozygotes, or suffers also from a slight defect in viability. Deliberate experiments on the viability of the different genotypes together with counts of the frequencies in nature would enable the inference that the dominants really exhibit the more advantageous patterns to be put to a quantitative test.

The incidence of dominance and linkage in the fish *Lebistes reticulatus* strongly suggests that the colored genes

found by Winge are advantageous in the male but disadvantageous in the female.

The association of the three peculiarities of polymorphism, close linkage and the universal recessive type of dominance is found in mollusks, arthropods and vertebrates. It is tentatively suggested that, at least in the grouse locusts and the snails, the primary cause of the two other phenomena may be found in the closeness of linkage within or between chromosomes. This condition presents an obstacle to normal evolutionary development by gene substitution, and so makes it possible for abnormalities such as duplications to possess occasional advantages, so setting up the stability of the gene-ratio necessary for polymorphism; if the advantage lies in the external appearance, the polymorphism will be manifest, and the variant form will tend to become dominant.

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631.48 : 561.5

*The Relationship of Climatic and Geological Factors to the Composition of Soil Clay and the Distribution of Soil Types.*

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Few soil problems have aroused more discussion and controversy than the assessment of the relative importance of climatic and geological factors in soil formation. At International Soil Congresses such as that held in 1927 in the United States it has been apparent that British and other workers familiar with small highly cultivated areas of irregular topography and varied geology attach much less importance to the climatic factors than do the Russians whose experience is largely of vast plains of fairly uniform loess material extending over well defined climatic zones. Purely practical considerations led under these extreme conditions to the development of geological and climatic systems of soil classification respectively, but both systems were found to require considerable modification when they were applied to other countries or when the scale of soil mapping was greatly changed. Even in the British Isles a generalised soil map would show the influence of climate, and detailed soil mapping by the Russian school would allow for considerable modifications of the climatic soil types by variations in local geology and topography.

Both systems are open, however, to the more fundamental criticism that they are based not on the actual properties of the objects classified but on external factors which have influenced the formation of the soil to varying and unknown degrees. The Russian work has demonstrated that an essential preliminary to all field and laboratory examination of soils should be the

recognition and separation of the soil profile down to the unaltered parent material into a series of distinct horizons of approximately uniform composition and mode of formation. These morphological studies, however, have not led to any system of soil classification capable of general application largely because they yield essentially qualitative and highly subjective information. There is, therefore, a great need at present time for the combination of exact information on the composition of soils on a profile basis with such mapping of similar soils as will enable the effects of geological, climatic, and human influences to be deduced by a rigid analysis of quantitative data. The present paper contains a preliminary attempt (1) to separate the influence of two climatic factors and a broad geological grouping on one of the most fundamental chemical characteristics of soils, the molecular ratio of silica to alumina in the clay fraction and (2) to examine the contribution of mean annual rainfall and temperature to soil formation by an examination of the distribution of recognised soil types. The method of analysis is applicable to other geographical and ecological problems in which it is necessary to assess the importance of several contributory factors some of which may be quantitative and others qualitative.

### *The Composition of Soil Clay.*

Chemical and physical analyses of soils have in the past contributed relatively little to our knowledge of the processes involved in the formation of soils and in the maintenance of their fertility. The failure may be ascribed partly to an excessive attention to the elements believed to have direct nutritive value to plants and partly to the separation of chemical from physical analyses. E. W. Hilgard's (1905) numerous comparisons of the acid extracts of soils from arid and humid regions demonstrated little beyond the accumulation of the commoner bases and acids near the surface in dry regions and of the finer soil particles in the subsoil in the humid districts. Although it has always been customary to discard as stones any particles larger than 2 or 3 mm. in diameter before analysing a soil, the desirability of making a physical fractionation at a much lower value of particle size has only recently been recognised in soil laboratories. It is apparently not yet appreciated by ceramic chemists and sedimentary petrologists, although they too have come to distrust the earlier attempts to distinguish by means of acid extractions or "rational analysis" between the essential product of weathering, and the coarser slightly weathered or adventitious material. Total analyses have now generally replaced acid extractions of soils but much of the information they provide

can be obtained more directly by simple physical analysis. Thus an accumulation of aluminium in a B horizon may arise from a purely mechanical washing down of fine particles. A combination of physical fractionation with chemical and mineralogical analyses of the fractions is desirable, but for many purposes a chemical analysis of the finest fraction of mechanical analysis proves sufficient. The precise value to be set as the upper limit of particle size for the weathering complex or colloidal material is difficult to determine but a compromise between theoretical requirements and laboratory convenience is afforded by the definition of the clay fraction in mechanical analysis adopted by the International Society of Soil Science (a settling velocity of less than 1.25 cm. per hour in water at 20° C., nominally a diameter of 2  $\mu$ ). In other words it is sufficient for the majority of investigations to regard the clay fraction of mechanical analysis as the inorganic colloidal material or weathering complex of the soil. Certainly there is no reason to postpone the investigation of this fundamentally important material on the grounds that its preparation requires the use of a supercentrifuge. It happens that many of the earlier investigations, including the ones to be considered here, were made on the so-called "ultra-clay," but there is every reason to believe that the clay fraction obtained by a satisfactory method of mechanical analysis would have given similar results. The exchangeable cations associated partly with this clay and partly with the organic colloids may be determined simply and directly from the total soil, and the results have proved of great value not only in agricultural advisory work but, as shown by K. K. Gedroiz (1929), for some aspects of soil classification. These cations are, however, so reactive and so easily modified by agricultural operations that for a study of the effects of climate and geology they are of less interest than the colloidal anions or clay.

The only systematic study of the colloidal clay of soils from a wide range of climatic and geological conditions is in the admirable work of W. O. Robinson and R. S. Holmes (1924) in the United States Bureau of Soils. Their data consist of full chemical analyses of the colloidal matter separated by dispersing the soil in water, and removing the material coarser than about 0.3  $\mu$  in diameter by settling and by high speed centrifuging. Soil from 30 centres in the United States was selected so as to represent the more important agricultural kinds of soil with a wide range of texture, geographical distribution, and conditions of soil formation, omitting only special soil types such as peats and laterites. At half of the centres samples were taken from two depths, but as these varied only slightly in the chemical properties considered here the deeper samples were omitted from the present analysis.

Robinson and Holmes made a cautious but restricted examination of the relationships between the values for mean annual rainfall and temperature at these centres, and the chemical composition of the 45 clays as exemplified by the molecular ratios  $\text{SiO}_2/\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3$  and  $\text{CaO} + \text{Na}_2\text{O}/\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3$ . They found undoubted but not close inverse relationships between both of these ratios and the rainfall, but they could trace none with the temperature. In general the colloidal matter developed in dry regions was richer in silica, lime, and soda than that in humid regions, but they concluded that there was insufficient evidence to justify the prediction of clay composition from the rainfall or even to show that the mature soils of any region had similar clays. The rainfall effect is to be expected for it is well known that soils of very wet districts, whether podsoles in cold ones or laterites in hot ones, have accumulation layers rich in sesquioxides, whilst desert soils generally have highly siliceous clays. This effect is well illustrated in F. J. Martin's (1929) collection of data from a belt across Central Africa. The mean  $\text{SiO}_2/\text{Al}_2\text{O}_3$  values for clay tractions grouped according to rainfall were 1.55, 2.07, 2.39, 3.65 for rainfalls of 10-25, 25-50, 50-80 and over 80 inches respectively.

It is of interest to examine more closely the alleged absence of a temperature effect on Robinson and Holmes' American clays.

#### *Clay Composition and Temperature.*

H. Jenny (1929) has recently re-examined Robinson and Holmes' data in an attempt to show that the more important climatic factors operating in soil formation may be estimated to a first approximation by Meyer's N.S. Quotient (mean annual rainfall in millimetres divided by the difference between the mean saturation pressure and the mean vapour pressure both in millimetres of mercury). He selected from the 30 centres 19 having approximately constant values of this function with the object of securing constant moisture conditions ("Befeuchtung"), and claimed that after rainfall differences are allowed for in this way the molecular ratio  $\text{SiO}_2/\text{Al}_2\text{O}_3$  falls off regularly with increasing temperature. This conclusion has already been quoted with approbation in a recent authoritative work of reference (H. Harrassowitz (1930))—"Jenny wies auch auf ausgezeichneten Zusammenhang des Quotienten  $\text{SiO}_2/\text{Al}_2\text{O}_3$  mit Jahrestemperatur in Nordamerika hin"), and it therefore seems necessary to point out that owing to the small variation in relative humidity in the 19 centres selected by Jenny the denominator of the N.S. Quotient is approximately a linear function of the temperature. Constancy of the N.S. Quotient therefore means that the rainfall increases regularly with

the temperature, and Jenny thus demonstrates nothing more than the rainfall effect originally recognised by Robinson and Holmes in more extensive and representative data. Actually the correlation of the  $\text{SiO}_2/\text{Al}_2\text{O}_3$  ratios with temperature in Jenny's selection of data is not significant, but if two erratic centres be arbitrarily discarded the negative correlation becomes significant. But as it happens to be accompanied by a much closer correlation between rainfall and temperature ( $r_{\text{RT}} = +0.86$  for 17 centres) the whole of the effect must be ascribed to rainfall, as originally concluded by Robinson and Holmes, and not to temperature. It happens, however, that a high positive correlation between rainfall and temperature ( $r_{\text{RT}} = +0.57$ ) is also found in Robinson and Holmes' 30 centres and explains their failure to recognise the temperature effect on clay composition.

The association of high values of rainfall and temperature is even more general, for data collected by Jenny from 145 meteorological stations distributed over 45 of the United States still give a highly significant positive correlation coefficient ( $r_{\text{RT}} = +0.43$ ). This general relationship not only obscures the effects of rainfall and temperature when tests are made by simple correlations, but brings out clearly one of the essential reasons for the different distributions of soil types in the United States and in Russia. Since evaporation is less at lower temperatures, leaching tends to increase with increase of rainfall and with decrease of temperature. Now in European Russia, where the connection between climatic and soil zones was first recognised, rainfall increases and temperature decreases from south to north. The two factors work in the same direction and leaching increases still more rapidly than rainfall and produces well-defined soil zones across the continent. But in the United States, at any rate in the areas where meteorological stations exist and soils have been studied, rainfall and temperature are positively correlated. Increasing temperature offsets some of the effect of increasing rainfall on drainage and leaching. Comparable leaching conditions may exist at widely different temperatures with consequent differences in native vegetation and in the rates of decomposition of soil organic matter. This not only smooths out soil boundaries and modifies the arrangement of soil zones but makes possible the production of soil types entirely different from those recognised and studied in Russia. It may be mentioned parenthetically in this connection that during the International Soil Excursion in the United States in 1927 one distinguished Russian pedologist on meeting an American prairie soil for the first time refused to accept a statement of the local rainfall on the grounds that it was quite impossible for it to produce such a soil.

*Statistical Examination of Clay Composition and Climatic Data.*

In view of the high correlation of rainfall and temperature in Robinson and Holmes' centres it is evidently desirable to make a fuller statistical examination of their data than has hitherto been attempted and R. A. Fisher's (1930) Analysis of Variance is obviously well suited for the purpose. The molecular ratio of  $\text{SiO}_2$  to  $\text{Al}_2\text{O}_3$  (S) was used in preference to the silica-sesquioxide ratio, for, although the two ratios happened to be closely proportional in the soils examined, there is some evidence in other work in this laboratory and elsewhere (*cf.* p. 19) that iron compounds move about in the soil independently of silica and aluminium compounds. The simpler ratio has the additional advantage that it suggests comparison with the alumino-silicates and alumina-silica gels which have been more thoroughly studied than the corresponding iron compounds. For similar reasons the CaO per cent. of the dry colloid (C) was used as a measure of the more mobile bases instead of the ratio  $\text{CaO} + \text{Na}_2\text{O}/\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3$ .

Table I gives these recalculated analytical data and the mean annual rainfall and temperature for all of Robinson and Holmes' soils, omitting the deeper soil where two samples were taken from the same profile. The principles underlying the geological grouping in Table I are discussed later.

The first stage of the analysis is the calculation of the regression coefficients of  $\text{SiO}_2/\text{Al}_2\text{O}_3$  and CaO per cent. respectively on rainfall and temperature. The significance of the regression equation is estimated by evaluating Fisher's statistic  $z$ , which is half the natural logarithm of the ratio of the variance of the values obtained from the regression equation to the remaining variance due to deviations from the equation. For  $\text{SiO}_2/\text{Al}_2\text{O}_3$  the value of  $z$  is well above that likely to arise by chance in 1 per cent. of a series of random samplings ( $P < 0.01$ ); for the CaO the value of  $z$  corresponds to  $P = 0.02$ . Both equations may, therefore, be taken as significant and subject to the standard errors (S.E.) given in Table II.

The decrease of  $\text{SiO}_2/\text{Al}_2\text{O}_3$  with increasing rainfall is thus confirmed but the effect of temperature on clay composition proves to be different from that concluded by either Robinson and Holmes who found no correlation, or Jenny who found a negative one. For constant rainfall clays become more siliceous with *increasing* temperature as would be expected from the effect of temperature on leaching. The relative importance of the rainfall and temperature effects on clay composition may be estimated by evaluating the standard errors of the individual regression coefficients and applying Fisher's  $t$  test. For both

Table I.

No.	Soil province or region.	Soil type.	State.	Depth in inches.	Rainfall in inches. R.	Temperature, ° F. T.	SiO <sub>2</sub> /Al <sub>2</sub> O <sub>3</sub> in colloidal clay.		CaO per cent. in colloidal clay.		
							Actual (S).	Calculated from S=S <sub>0</sub> -0.0791 R+0.0699 T	Actual (C).	Calculated from C=C <sub>0</sub> -0.0676 R+0.0544 T	
A. Igneous and Metamorphic.											
4	I	Cecil clay loam	Georgia	0-9	49	62	1.69	2.08	0.31	0.52	
19	I	Iredell clay	N. Carolina	6-24	52	64	2.11	1.99	0.61	0.43	
6	I	Chester loam	Virginia	0-7	44	55	2.12	1.99	0.17	0.47	
21	I	Manor loam	Maryland	0-7	40	54	2.12	2.24	0.64	0.69	
3	I	Cecil clay loam	Maryland	0-7	40	53	2.15	2.17	0.51	0.64	
7	I	Chester loam	Maryland	0-8	43	53	2.21	1.93	0.93	0.43	
B. Limestone.											
42	(?)	Vega Baja clay loam	Porto Rico	0-12	69	77	1.87	2.00	0.44	0.43	
14	II	Hagerstown loam	Maryland	0-12	40	54	2.34	2.68	1.25	1.14	
9	II	Clarks ville loam	Kentucky	0-10	49	58	2.72	2.25	0.62	0.75	
C. Marine Deposits from Igneous and Metamorphic.											
40	III	Susquehanna clay	Mississippi	0-4	58	65	1.70	2.26	0.20	0.43	
27	III	Norfolk fine sandy loam	N. Carolina	0-8	50	52	2.08	1.98	0.54	0.26	
31	III	Orangeburg fine sandy loam	Mississippi	0-10	53	63	2.21	2.51	0.51	0.66	
33	III	Sassafras silt loam	Maryland	0-8	41	55	2.32	2.90	0.75	1.03	
41	III	Susquehanna clay	Maryland	60-72	41	53	2.61	2.76	0.24	0.93	
20	III	Lufkin clay	Mississippi	5-36	52	64	4.15	2.66	1.85	0.78	

Table I—(continued).

No.	Soil province or region.	Soil type.	State.	Depth in inches.	Rainfall in inches R.	Temperature, °F. T.	SiO <sub>2</sub> /Al <sub>2</sub> O <sub>3</sub> in colloidal clay.		CaO per cent. in colloidal clay.		
							Actual (S).	Calculated from $S=S_0-0.0791 R+0.0699 T$	Actual (C).	Calculated from $C=C_0-0.0676 R+0.0544 T$	
D. Marine Calcareous Clay.											
11	III	Crowley silt loam	Louisiana	0-10	55	68	2.91	3.07	0.99	2.51	
16	III	Houston black clay	Texas	0-12	38	65	4.36	4.20	5.01	3.49	
E. Glacial and Loessial.											
12	IV	Dunkirk clay loam	New York	0-8	40	45	2.62	2.49	0.63	0.59	
29	V	Ontario loam	New York	0-12	34	47	2.77	3.10	1.37	1.10	
25	V	Miami silty clay loam	Indiana	0-40	37	50	3.29	3.08	0.92	1.06	
1	V	Carrington loam	Iowa	0-12	32	47	3.37	3.26	1.48	1.24	
23	V	Marshall silt loam	Nebraska	0-14	30	51	3.59	3.70	1.19	1.59	
F. Alluvium from Igneous.											
17	VI	Huntington loam	Maryland	0-8	35	53	2.37	2.61	0.70	0.36	
38	VII	Stockton clay adobe	California	0-38	28	62	3.73	3.79	1.96	1.33	
37	VII	Stockton clay adobe	California	0-12	14	60	3.86	4.76	1.47	2.16	
45	VII	Yolo clay	California	0-18	20	58	4.78	4.14	1.46	1.85	
13	VIII	Fallon loam	Nevada	0-12	5	51	5.41	4.84	2.20	2.28	
G. Alluvium from Loess.											
36	VI	Sharkey clay	Mississippi	0-4	54	66	3.85	3.90	1.41	1.64	
35	VI	Sharkey clay	Mississippi	?	54	66	4.15	3.90	1.92	1.64	
43	VI	Wabash silt loam	Nebraska	0-15	34	52	4.29	4.50	2.19	2.23	

List of soil provinces or regions.

- |     |  |      |                                |
|-----|--|------|--------------------------------|
| I   | Piedmont Plateau Province.                 | V    | Glacial and Loessial Province. |
| II  | Limestone Valleys and Uplands Province.    | VI   | River Flood Plains Province.   |
| III | Atlantic and Gulf Coastal Plains Province. | VII  | Pacific Coast Region.          |
| IV  | Glacial Lake and River Terrace Province.   | VIII | Great Basin Region.            |



Table II.—Regression Equations for Clay Composition on Rainfall and Temperature. (Ungrouped data.)

R = Mean annual rainfall in inches.

T = Mean annual temperature in ° F.

S = Molecular ratio of  $\text{SiO}_2$  to  $\text{Al}_2\text{O}_3$  in clay.

C = CaO per cent. in clay.

SE = Standard error.

	Regression equations.			Regression coefficient.	
	SE.	z.		SE.	t.
$S = 2.053 - 0.0647 R + 0.0626 T$	0.0736	1.304	S on R	0.0124	5.22
$C = 0.405 - 0.0441 R + 0.0586 T$	0.836	0.812	S on T	0.0224	2.80
			C on R	0.0141	3.12
(For P = 0.01)		(0.851)	C on T	0.0254	2.31
					(2.77)

measures of clay composition the negative regression coefficient on rainfall considerably exceeds the value to be expected by chance in 1 per cent. of a series of samplings; the positive regression coefficients on temperature are not so well established but might arise by chance in 1 per cent. for  $\text{SiO}_2/\text{Al}_2\text{O}_3$ , and in 3 per cent. for CaO. Variations of rainfall therefore are more important than those of temperature, but even over the moderate range covered by these data the temperature effect is highly significant. From the ratio of these two regression coefficients it would follow that the  $\text{SiO}_2/\text{Al}_2\text{O}_3$  ratio would remain constant when an increase of 1° F. is accompanied by an increase in rainfall of 0.97 inches and that CaO per cent. would be constant for an increase in rainfall of 1.33 inches per ° F. A more detailed consideration of these ratios will be given later.

#### *Relation of Clay Composition to Geology.*

A correlation of clay composition with rainfall and temperature might easily arise from the dependence of climate on topography and geology. The intimate association of climate and geological factors in soil formation has been overlooked almost as frequently as the association of rainfall and temperature already discussed. In the British Isles the complexity of the geological factors influences soil formation and distribution not merely by affording a wide range of rocks for parent material and a highly irregular surface which

prevents a full soil development but also by an indirect action on the climate. Low rainfall areas occur in areas of tertiary and more recent formations ; areas of primary formations generally have high rainfalls and low temperatures. The separation of geological from climatic effects is therefore necessarily difficult but the attempt must be made before the fundamental principles of soil genesis and classification can be placed on a firm and quantitative basis. Robinson and Holmes found the influence of parent material more difficult to estimate than the rainfall effect. They attempted no analysis, but merely illustrated the influence of the age of the soil by referring to the high  $\text{SiO}_2/\text{Fe}_2\text{O}_3 + \text{Al}_2\text{O}_3$  ratios in two soils which are relatively young in the localities where they are now found, and the low values of a group of 10 more mature soils which happen, however, to occur together in a region of relatively constant climatic conditions. They give no other observations on the maturity of the soils as manifested in morphological characteristics and as deduced from topography, and also no information about the geological formations from which the soils were derived. Fortunately the latter omission can be remedied to some extent by using the names of the soil types in conjunction with the information contained in the valuable Bulletin 96 of the U.S. Bureau of Soils. This describes the soils of the United States with the system of classification adopted in 1913. There are 13 main sub-divisions, six soil regions in the Great Plains and the country to the west and seven soil provinces in the east. These are differentiated on general geographical features rather than on soil characters and are consequently inadequate for an attempt to separate climate and geology. Provinces and regions are divided into soil series of the same colour, subsoil, and origin, and these in turn are divided into soil types which have the same texture, colour, structure, subsoil, topography, process of derivation and parent material. Sufficient information is given in Bulletin 96 to justify a classification of the 30 soils sampled by Robinson and Holmes into seven geological groups according to the process of formation of the parent material of the soil. Owing to the small number of samples the grouping is necessarily rough and to restrict the number of groups it is essential to put together such related formations as the various glacial and loess deposits. The geological grouping and the soil regions or provinces are given in Table I and the mean clay compositions within the geological groups are given in Table V, together with certain quantities deduced from the data in Table I.

R. A. Fisher's "Analysis of Variance" provides a ready means of testing (1) the efficiency of this geological grouping ; (2) the significance of the regressions on rainfall and temperature after the effects of this geological grouping

have been eliminated ; and (3) the significance of the geological effects after elimination of the average effects due to rainfall and temperature.

Table III.—Analysis of Variance for Geological Grouping.

—	Degrees of freedom.	S ( $\text{SiO}_2/\text{Al}_2\text{O}_3$ ).		C (CaO) per cent.	
		Sum of squares.	Standard error.	Sum of squares.	Standard error.
Total .....	29	29.289	—	25.942	—
Between geological groups .....	6	17.891	—	13.179	—
Within geological groups .....	23	11.398	0.704	12.763	0.745
(For $P = 0.01$ , $z = 0.656$ ) .			( $z = 0.897$ )		( $z = 0.688$ )

Table III shows that the grouping on a geological basis has proved even more efficient than the regression equations on climatic factors as a means of estimating the clay composition from external conditions (for  $\text{SiO}_2/\text{Al}_2\text{O}_3$  the standard error for the regression on R and T is 0.736 and for the geological grouping 0.704).

*Relation of Clay Composition to Geology and Climate.*

At this stage the analysis serves to illustrate an impasse often encountered in soil classification and in other geographical and ecological fields. Here and elsewhere where the properties to be investigated can be expressed quantitatively, the qualitative geological grouping and the regression on climatic factors can be combined in the analysis. The two variances for “between groups” and “within groups” are each further divided into a fraction expressed by average regression equations on rainfall and temperature and a remainder from which a final standard error of the combined analysis is obtained (Table IV). In both measures of clay composition the regression

Table IV.—Regression Equations for Clay Composition within Geological Groups on Rainfall and Temperature.

( $S_0$  and  $C_0$  are constants for each geological group.)

—	Regression equation.		—	Regression coefficients.	
	SE.	z.		SE.	t.
$S = S_0 - 0.0791 R + 0.0699 T$ ..	0.512	1.197	S on R	0.0063	12.52
			S on T	0.0093	7.52
$C = C_0 - 0.0676 R + 0.0544 T$ ...	0.633	0.848	C on R	0.0210	3.22
(For $P = 0.01$ ) .....		(0.877)	C on T	0.0308	1.77
					(2.83)

equations for "between groups" are not significant, *i.e.*, the difference between the geological groups are independent of the mean values of rainfall and temperature for those groups. It follows, therefore, that within the geological groups the regression equations are more efficient than those derived from the original ungrouped data and that the association of clay composition with climatic factors is not a chance effect due to a common dependence on geology. The validity of the average climatic effects is placed on a firmer basis and the clay composition may be estimated from the geological grouping and average regressions on rainfall and temperature with standard error of 0.51 for  $\text{SiO}_2/\text{Al}_2\text{O}_3$  and 0.63 for CaO per cent.

The values of  $\text{SiO}_2/\text{Al}_2\text{O}_3$  and CaO per cent. calculated from these regression equations are compared with the actual values in Table I and the  $\text{SiO}_2/\text{Al}_2\text{O}_3$  ratios are given graphically in fig. 1 together with the constants  $S_0$  for the geological groups. When it is remembered that no special care had been taken in the collection of soil samples to secure centres in which the soils were fully developed and in stable equilibrium with the climate, it will be seen that the combination of a geological grouping with the simplest climatic factors accounts for a large fraction of the total variation in clay composition. A few of the more erratic soils will be referred to subsequently. The improved fitting obtained by eliminating some of the geological effects improves the accuracy and utility of the regression coefficients on rainfall and temperature, especially for  $\text{SiO}_2/\text{Al}_2\text{O}_3$ , and in the remainder of this paper the regression coefficients in Table IV will be used in preference to those for the ungrouped data in Table II.

The regression equations provide constants for each geological group which are independent of the climatic conditions, *viz.*, the quantities ( $S_0$  and  $C_0$ ) corresponding to conditions in which the negative rainfall effect equals the positive temperature effect. These values are tabulated in Table V in order of increasing values of  $S_0$  and show that, whereas for both the groups of alluvial soils the mean value of  $\text{SiO}_2/\text{Al}_2\text{O}_3$  exceeds 4.0, these high values have been produced by quite different forces. For alluvium from igneous and metamorphic rocks the geological factor is not significantly greater than that for the residual soils from similar rocks; the high value is to be ascribed to weathering in an arid climate. On the other hand the alluvium from loess has the highest geological factor of the series and a relatively low climatic factor. It is clear from these extremes that attempts to relate clay composition to climatic factors must involve a preliminary elimination of geological factors either by some such method as the above or by a careful selection of uniform parent

Table V.—Mean Values of  $\text{SiO}_2/\text{Al}_2\text{O}_3$  and  $\text{CaO}$  per cent. in Clay for Geological Groups.

Climatic factor for  $\text{SiO}_2/\text{Al}_2\text{O}_3 = 0.0699 \text{ T} - 0.0791 \text{ R}$ .  
 Climatic factor for  $\text{CaO}$  per cent.  $= 0.0544 \text{ T} - 0.0658 \text{ R}$ .  
 Geological factor = mean of group — climatic factor.

Geological group.	Igneous and meta-morphic.	Alluvium from igneous.	Lime-stone.	Marine deposits from igneous.	Glacial and loessial.	Marine deposits (calcareous clays).	Alluvium from loess.
Number of soils . . . . .	6	5	3	6	5	2	3
Mean of rainfall in inches . . . . .	44.7	20.4	52.7	49.2	34.6	46.5	47.3
Mean temperature in ° F. . . . .	56.8	56.8	63.0	58.7	48.0	66.5	61.3
<i><math>\text{SiO}_2/\text{Al}_2\text{O}_3</math> in clay.</i>							
Actual mean, $\text{Sg}$ . . . . .	2.07	4.03	2.31	2.51	3.13	3.64	4.10
Geological factor, $\text{Sg}$ . . . . .	1.63	1.67	2.07	2.30	2.51	2.67	3.56
Climatic factor . . . . .	0.44	2.36	0.24	0.21	0.62	0.97	0.54
Standard error . . . . .	0.21	0.23	0.30	0.21	0.23	0.36	0.30
<i><math>\text{CaO}</math> per cent in clay.</i>							
Actual mean, $\text{Cg}$ . . . . .	0.53	1.56	0.77	0.68	1.12	3.00	1.84
Geological factor, $\text{Cg}$ . . . . .	0.46	—0.15	0.90	0.81	0.85	2.53	1.70
Climatic factor . . . . .	0.07	1.71	—0.13	—0.13	0.27	0.47	0.14
Standard error . . . . .	0.26	0.28	0.37	0.26	0.28	0.45	0.37

material or alternatively for fully developed soils by a demonstration that the clay composition is independent of geological factors.

*The Ratio of Silica to Alumina in Soil Clay.*

Although the data are insufficient to justify detailed discussion of the individual geological factors the results in Table V suggest that under comparable climatic conditions  $\text{SiO}_2/\text{Al}_2\text{O}_3$  increases steadily with increasing reworking and water transport of the parent material. The lowest values of the geological factors are for soils derived from igneous rocks either directly as sedentary soils or indirectly with one stage of transport for relatively short distances by mountain rivers. Intermediate geological factors are given by the Tertiaries of the Atlantic and Gulf Coastal Plains which are known to have been derived from igneous and metamorphic rocks and also by the glacial and loessial material much of which had undoubtedly been transported in water before and after glaciation. The most siliceous clays are for the alluvium carried by the Mississippi River and its tributaries from the loessial material of the Great Plains. It is not possible to decide from the present data whether the high  $\text{SiO}_2/\text{Al}_2\text{O}_3$  ratios of the clay fractions of soils derived from relatively recently reworked deposits depend on the greater immaturity of the soils or on the changes in minerals and colloidal material produced by the reworking. Until further information is available on the degree of development of the soil profiles and the thoroughness of the dissection of their physiographical regions, it would appear simpler to summarise the influence of the geological nature of the parent material in the statement that transport and weathering in rivers and coastal waters returns to the clay, or to material weathering to clay in the soil, some of the silica that is removed from it by the processes of weathering in the acid soils of humid regions. In arid regions the clays remain highly siliceous in both mature and young soils presumably because they are formed and remain in the presence of relatively large amounts of soluble silicates in alkaline solutions.

The recognition of two dissimilar sources of highly siliceous clays accounts for some of the unexpected results contained in the earlier clay analyses and for some of the irregularities in the present data. Thus in the earliest British clay analyses, A. D. Hall, and E. J. Russell (1911) found several clays in South-East England with  $\text{SiO}_2/\text{Al}_2\text{O}_3$  ratios above 4.0. These values are comparable with those obtained later by A. F. Joseph (1924) in the more arid parts of the Sudan. Such similarities in widely different regions appear irreconcilable with important climatic effects until it is realised that many of

Hall and Russell's soils were derived from tertiary and recent formations repeatedly reworked in water. In Scotland and North Wales the high leaching and the great age of the geological formations combine to give low  $\text{SiO}_2/\text{Al}_2\text{O}_3$  ratios in the clays, though here too there is some indication that glacial drift soils have higher  $\text{SiO}_2/\text{Al}_2\text{O}_3$  ratios than sedentary ones. G. W. Robinson (1928) in advancing the hypothesis that the primary product of the chemical weathering of mineral silicates is a mixture of kaolinite and nontronite (or of other hydrated silicates with silica-sesquioxides ratio of 2.0) has already suggested that the clay complex of estuarine and other littoral sediments is enriched in silica by the concomitant precipitation of the silicic acid present in river water. He gives for the mean values of the silica-sesquioxide ratio of the clay fractions of North Wales soils 1.90 for those derived from crystalline or consolidated rocks and 2.67 for those derived from alluvium or unconsolidated sediments.

Again, in a few early analyses by W. T. McGeorge (1917) of Hawaii soils the clay fractions from areas of very high rainfall had much lower  $\text{SiO}_2/\text{Al}_2\text{O}_3$  ratios (0.4, 0.7) than from those with moderate rainfall ( $\text{SiO}_2/\text{Al}_2\text{O}_3 = 1.3, 1.6, 1.7, 1.7, 1.9, 2.4$ ), but it happens that the most siliceous clay ( $\text{SiO}_2/\text{Al}_2\text{O}_3 = 3.0$ ) is from a soil which differs completely from the others and resembles the highly plastic clay adobe soils of California. This soil is found only in gullies and valleys and is clearly derived from transported material weathered in contact with highly siliceous waters. The grosser discrepancies between the actual and calculated values in Robinson and Holmes' data in Table I and fig. 1 may be accounted for in a similar way. In the humid regions the Lufkin clay which has a higher  $\text{SiO}_2/\text{Al}_2\text{O}_3$  than that calculated happens to be from a very raw unweathered subsoil of marine sediment in which the geological effect still masks the climatic one. In the arid region the Stockton clays, one of which has a much lower  $\text{SiO}_2/\text{Al}_2\text{O}_3$  than that calculated, were formed under bad drainage conditions, and have probably been subjected to more leaching than is indicated by their present rainfalls. Allowance for this earlier leaching would make the regression lines fit more closely and yield a geological factor for igneous alluvium appreciably above that for the residual igneous material.

The joint action of geological and climatic factors may alternatively be described in the statement that young soils derived from relatively recent reworked deposits tend to have more siliceous clays than those of old soils derived directly from igneous and metamorphic rocks. It is not, of course, possible to decide from this analysis whether the prime product of weathering

in the soil is a colloidal material with a composition approximating to  $5 \text{ SiO}_2$ ,  $1 \text{ Al}_2\text{O}_3$ , stable in the alkaline conditions of arid regions but breaking down progressively to  $2 \text{ SiO}_2$ ,  $1 \text{ Al}_2\text{O}_3$  with leaching in acid soils, or whether the latter is formed first and becomes more siliceous by contact with relatively concentrated alkaline silicate solutions in arid soils or with dilute silicate solutions in prolonged river transport. The facts that the highly siliceous material is stable in arid regions and the less siliceous one is known to break down still further to laterites and bauxite under intense leaching at high temperatures, point to the more siliceous clay as the primary product of weathering. In any case the present analysis suggests that colloidal clay may be regarded as a mixture of two forms, which may even be present as a core and coating in the same particle, the one determined by the parent geological material and the other by the climatic conditions in which the clay was formed. In young soils the geological and in old soils the climatic effect predominates. The separation of effects due to the age of a soil from those due to the mineral composition of its parent material presents a more complex problem, but one which is capable of treatment by the above methods when a sufficient body of independent data is available.

#### *Variation of Clay Composition in the Soil Profile.*

After the rest of the present paper had been prepared for publication a further communication from the U.S. Bureau of Chemistry and Soils was received in which I. A. Denison (1930) gave the results of a detailed examination of the clay fractions of samples taken on a profile basis from seven soils selected as representing four widely different groups of soils and with different parent materials and degrees of maturity of the soil within one of the groups. Although the number of soils is not sufficient to distinguish between the soil age and mineralogical composition, it is of interest to examine the extent to which the conclusions already drawn facilitate the interpretation of profile data. Table VI gives Denison's data recalculated to  $\text{SiO}_2/\text{Al}_2\text{O}_3$  ratios and with the horizons of the profiles grouped on a common basis (A is a leached surface layer, B an accumulation layer, and C partially modified parent material. For ease of comparison in one soil the uppermost of three C horizons is treated as a B horizon since no B horizon was analysed, and the description is not inconsistent with this grouping). No climatic data were given, but sufficiently accurate values were obtained from atlases to justify the calculation of approximate climatic factors from the regression equation for  $\text{SiO}_2/\text{Al}_2\text{O}_3$  on



Table VI.—Clay Composition of Profile Horizons (Denison's data).

Number	7	1	6	4	5	2	3
Soil type	Tschernosem	Podsol	Rendzina	Red soil Immature Gneiss	Red soil Partial Granite	Red soil Full Gneiss	Red soil Full Mica schist
Profile development	Glacial drift	Glacial drift	Marl	—	—	—	—
Parent material	1-3	0-7	0-7	-0-7	+0-1	-0-7	+0-1
Climatic factor for $\text{SiO}_2/\text{Al}_2\text{O}_3$							
<i><math>\text{SiO}_2/\text{Al}_2\text{O}_3</math> in clay fraction.</i>							
Horizon A	4-53	3-55	2-95	1-75	1-86	1-82	1-58
" B	4-34	3-44	2-85	1-70	(C <sub>1</sub> ) 1-65	1-59	1-39
Upper C	—	—	—	—	1-02	1-25	1-86
Lower C	5-06	5-33	3-10	1-76	0-91	1-20	2-08
<i><math>\text{Fe}_2\text{O}_3/\text{Al}_2\text{O}_3</math> in clay fraction.</i>							
Horizon A	0-39	0-21	0-22	0-19	0-11	0-23	0-22
" B	0-41	0-40	0-21	0-14	(C <sub>1</sub> ) 0-07	0-25	0-25
Upper C	—	—	—	—	0-04	0-17	0-29
Lower C	0-43	0-56	0-21	0-14	0-04	0-08	0-15

rainfall and temperature given in Table IV. (The climatic data for the hilly districts of the red soils are the least accurate.)

The A horizons, presumably owing to their greater acidities and organic matter contents, have consistently higher  $\text{SiO}_2/\text{Al}_2\text{O}_3$  ratios than the B horizons. (Difference 0.14, standard error 0.025.) The  $\text{SiO}_2/\text{Al}_2\text{O}_3$  of the A and B horizons are much less variable than those of the C horizons, especially in the groups of Red Soils. The true soil horizons thus show a closer approach to equilibrium with the climate. There is a significant correlation ( $P = 0.01$ ) between the clays of either the A or the B horizons and the climatic factors, but the B clay is also significantly correlated with the C clay. {The composition of the B clay may be expressed satisfactorily ( $P = 0.01$ ) by an equation of the type

$$\begin{aligned} \text{SiO}_2/\text{Al}_2\text{O}_3 \text{ of B} = & 1.28 + 0.41 (\text{SiO}_2/\text{Al}_2\text{O}_3 \text{ of C}) \\ & + 0.53 (\text{Climatic Factor}). \end{aligned}$$

The C clays are clearly more influenced by the geological formations than the A or B clays, but even the C clays show some correlation with climate and cannot, therefore, be taken as independent measures of the contribution of the geological factors to the soil composition. In other words the C material is not a primary weathering product independent of climate. This is explained when the age of the soils and maturity of the profiles are considered. Two of the soils are immature. The Rendzina (No. 6) is a young soil in which the development of the profile has been retarded by its heavy texture and high calcium content and one of the red soils (No. 4) was definitely selected on morphological and topographical evidence as being immature. In both of these, the  $\text{SiO}_2/\text{Al}_2\text{O}_3$  of the C clay is similar to that of the A clay which is in fact but slightly altered C material. The three other Red Soils are extremely old, and it would appear that the whole profile has been influenced by soil forming processes with an accumulation of alumina relative to silica even in the horizon of partially disintegrated rock. The influence of the mica in the parent rock is, however, still to be found in the C clay of soil No. 3.

The higher  $\text{SiO}_2/\text{Al}_2\text{O}_3$  ratios in the C material of the recent reworked sediments is in complete agreement with the conclusions already drawn from Robinson and Holmes' data. The Tschernosem (No. 7) and the Podsol (No. 1) are derived from similar glacial drift materials and in regions of the same temperature; the greater accumulation of the aluminium relative to silica in the A and B clays of the Podsol is in accordance with the higher rainfall and leaching in which they have been formed.

It may be noted also that although the American Bureau of Soils invariably quotes  $\text{SiO}_2/\text{Al}_2\text{O}_3 + \text{Fe}_2\text{O}_3$  ratios in preference to  $\text{SiO}_2/\text{Al}_2\text{O}_3$  ratios, an examination of the  $\text{Fe}_2\text{O}_3/\text{Al}_2\text{O}_3$  ratios supports the choice of the simpler ratio in this paper. The relative distribution of iron and aluminium within the profile is itself markedly influenced by climate and geology, and it may well prove to be more closely connected with the soil morphology than the ratio of silica to one or both of the sesquioxides.

Here as in other respects, the immature Rendzina shows no sign of segregation of clay constituents. The four red soils from igneous rocks have very low  $\text{Fe}_2\text{O}_3/\text{Al}_2\text{O}_3$  values in the lower C clay, with higher values in the A material in all cases, and still higher values in the B or upper C horizons in the two fully developed soils. There is thus distinct evidence of accumulation of iron relative to aluminium in the course of soil formation at relatively high rainfalls and temperatures. The glacial drift soils on the other hand have high  $\text{Fe}_2\text{O}_3/\text{Al}_2\text{O}_3$  ratios in the C material and progressively lower values in the B and A horizons, especially in the highly leached Podsol. Such indications of a differential effect of climatic and associated vegetational factors emphasise the advantage of considering iron and aluminium separately. The iron is probably influenced to a much greater extent than aluminium by the decomposition of soil organic matter through either reduction or the formation of co-ordination compounds.

#### *The Relative Effects of Rainfall and Temperature on Drainage.*

The examination of the influence of the mode of formation of parent material has demonstrated that the correlations found between  $\text{SiO}_2/\text{Al}_2\text{O}_3$  or CaO per cent. of the clay fraction, and rainfall and temperature do not arise from a chance association of geological and climatic factors within the small number of centres available. On the other hand it has shown that the elimination of these geological effects improves the accuracy of the relationship between clay and climate. The statement that for soils from similar parent materials a rise of  $1^\circ \text{F}$ . requires an additional 0.88 inches of rain to maintain constant  $\text{SiO}_2/\text{Al}_2\text{O}_3$  or of 0.80 inches for constant CaO per cent. in soil clay suggests that these factors measure the average effect of temperature and associated factors on leaching. It therefore becomes of interest to examine more directly the relative effects of rainfall and temperature on the drainage through soil. A sufficient number of lysimeters is not available and the measurements of evaporation made for plant physiological or engineering

purposes from free water surfaces or from various forms of atmometer bear little relation to the water losses from soil which may remain low for long dry periods even in atmospheres of high evaporating powers. Some substitute for a series of lysimeters in different climates may be obtained by examining the mean monthly values at a single centre. The three Rothamsted gauges are well adapted for examining the effect of temperature since the rainfall is fairly uniformly distributed throughout the year. The extreme values of monthly rainfall differ by only 36 per cent. of the mean value, and although there is a relatively dry spell in spring and summer the correlation between mean monthly rainfalls and temperatures is quite negligible. The three gauges are 1/1000 acre in area, 20, 40, and 60 inches deep respectively, and are kept free from vegetation. They were built round undisturbed blocks of a heavy loam on clay, and, as temperature records were not taken until they had been in operation for 7 years, irregularities due to drying and soil movements during their construction are eliminated by taking the mean values for the 51 years with full data. As the three gauges give similar results except for slight systematic differences probably due to irregularities in the distribution of stones and channels within the soil blocks the mean values for the three gauges were used. The data and statistical analysis are given in Table VII. The reduction of drainage by high summer temperatures is so great ( $r_{DT} = -0.77$ ,  $P < 0.01$ ) that it entirely masks the rainfall effect ( $r_{DR} = +0.47$ ,  $P = 0.1$ ). Both partial correlation coefficients ( $r_{DTR} = -0.97$  and  $r_{DR T} = +0.94$ ) are, however, highly significant and together account for a variance of 5.080 out of a total of 5.323. The significance of these relationships is shown even more clearly by the  $z$  test of the regression equations and the  $t$  test of the individual regression coefficients given in Table VII. The results become more comparable with those already derived from the examination of American clays if 12 times the mean monthly values of drainage and rainfall be regarded as equivalent to mean annual values at the appropriate temperature. The regression coefficients would then become  $+1.112$  inches of drainage per inch of rain and  $-0.834$  inches of drainage per degree Fahrenheit. To maintain constant drainage a temperature rise of  $1^{\circ}$  F. must be accompanied by 0.75 inches more rain. This agrees closely with the values 0.88 inches and 0.80 inches per  $^{\circ}$  F. required to maintain constant  $\text{SiO}_2/\text{Al}_2\text{O}_3$  and  $\text{CaO}$  per cent. respectively in Robinson and Holmes' 30 clays and serves to confirm the interpretation of the effects on clay of rainfall and temperature in terms of drainage and leaching.

Table VII.—Rothamsted Drain Gauge Data. Mean Monthly Values for Average of 3 gauges (20 inches, 40 inches and 60 inches deep) for 51 years, 1878–1928.

	Rainfall, R.	Temperature, T.	Drainage.	
			Actual, D.	Calculated from R and T.
	inches.	° F.	inches	inches
January .....	2·303	37·5	2·039	1·87
February.....	2·112	38·7	1·686	1·58
March .....	2·072	41·1	1·209	1·37
April .....	2·016	45·5	0·717	1·00
May .....	2·052	51·9	0·539	0·59
June .....	2·253	57·0	0·604	0·46
July .....	2·681	60·6	0·730	0·59
August .....	2·774	59·8	0·796	0·85
September .....	2·322	55·6	0·793	0·64
October .....	3·131	48·6	1·820	2·02
November ....	2·745	42·1	2·105	2·05
December .....	2·866	38·5	2·509	2·43
Mean .....	2·444	48·1	1·296	1·30

Regression Equations of Drainage on Rainfall and Temperature. (Mean Monthly Values.)

	Regression equations.				Regression coefficients.		
	SE.	z found.	z for P=0·01.		SE.	t found.	t for P=0·01.
$D = -0·821 + 0·866 R$ .....	0·644	0·518	1·333	D on R	0·516	1·68	3·17
$D = 4·282 - 0·0621 T$ .....	0·462	1·603	1·333	D on T	0·016	3·87	3·17
$D = 1·920 + 1·112 R - 0·0695 T$ ...	0·164	2·100	1·154	D on R	0·133	8·37	3·25
				D on T	0·006	12·04	3·25

*Climatic Factors in Relation to the Distribution of Soil Types.*

The possibility of accounting for considerable fractions of the variation in clay composition and drainage in terms of simple regressions on annual rainfall and temperature suggests the desirability of examining the consistency of these and other associated climatic factors for regions known to have similar soil types, and of attempting to deduce quantitatively the interaction of rainfall and temperature on soil formation from the actual distribution of soils. There

has been general agreement that neither rainfall nor temperature alone is sufficient to characterise the soil forming action of the climate and several attempts have been made to allow for the variation of evaporation with temperature. Most interest had been aroused by Lang's "Regenfaktor" (rainfall in centimetres  $\div$  temperature in degrees C.) and Meyer's "N.S. Quotient" (rainfall in centimetres  $\div$  absolute saturation deficit in millimetres Hg). Both are open to obvious objections especially for cold regions where the factor is necessarily high. The evidence advanced to support them has generally been limited to data from a few isolated centres and to the demonstration that maps of the distribution of the function over Europe and U.S.A. bear some superficial resemblance to maps of the broadest soil divisions. Recently Jenny (1930) has attempted a more exact treatment. He tabulated the relevant data from 65 meteorological stations in U.S.A. and grouped them into eight soil regions from soil maps prepared by C. F. Marbut (1928), the Head of the United States Soil Survey. Jenny claimed that the more complex function of Meyer is more accurate than that of Lang when judged by the range between the extreme values found within each group of soils. As this criterion is inadequate these functions and certain related values were examined by applying the  $z$  test to Jenny's data. With an efficient climatic function the variance between the soil groups should be high in comparison with the variance within the soil groups. (To avoid over-emphasising the group of yellow and red soils which contained twice as many soils as any other group in Jenny's collection of data the 19 centres were reduced to 10 by discarding alternate centres when arranged in order of increasing temperature.)

The values of  $z$  given in Table VIII show that although Meyer's "N.S. Quotient" is slightly better than Lang's "Regenfaktor," neither has any advantage over the rainfall or temperature alone as a means of grouping the

Table VIII.—Efficiency of Grouping of Climatic Factors by Grouping Centres according to Soil Properties.

(56 centres divided into 8 soil groups, Jenny's collection of data based on Marbut's maps.)

$z$	Climatic Factor.
2.28	Vapour Pressure in millimetres (V.P.) — calc. from T. and R.H.
2.17	Rainfall in centimetres (R).
2.12	Saturation Vapour Pressure in millimetres Hg (P) — calc. from T.
2.04	Temperature in ° C. (T).
1.92	Meyer's "N.S. Quotient" $[R/P (1 - RH)]$ .
1.73	Lang's "Regenfaktor" $(R/T)$ .
1.63	Relative Humidity (RH).
1.59	Saturation Deficit $[P (1 - RH)]$ .

climatic values in accordance with the soils. Further the arrangements of the soil groups in order of climatic values is unsatisfactory. Although Lang's and Meyer's functions agree in assigning the extremes of climates to grey desert soils and podsoils, they place the climate of the prairie soils with those of the highly leached red soils, yellow soils and iron laterites; Lang's factor assigns an even wetter climate to the Northern Tschernosem which Marbut has classed among the Pedocals or soils of arid climates. Such failures destroy the value of these combined climatic functions.

The relative humidity which has been proposed in Russia (Kaminski, 1924) as an improvement on these two factors, arranges the soil types roughly in the order expected from their compositions, but the  $z$  test shows that it is inefficient for grouping. The mean vapour pressure has proved the most effective single criterion for separating groups, but the arrangement is naturally the same as for temperature and is not in accordance with the soil composition. The mean vapour pressure has the advantage over Meyer's factor that it represents a simple physical quantity whereas the N.S. Quotient has meaningless dimensions, but it is rarely determined directly and its indirect calculation requires data not generally available. The mean annual rainfall thus remains the most useful single criterion for climatic grouping and mean annual temperature is almost as efficient.

An examination of the relationship of rainfall to temperature within the soil groups shows the reasons for the failure of the combined climatic functions. When the means of the groups are arranged in order of increasing rainfall the regression coefficients of rainfall on temperature within the groups increases from insignificant values in the drier regions to high positive values for the Prairie Soil and Podsol regions, and falls again to low values in the very humid regions of Red Soils and Ferruginous Laterites. This regression coefficient may be expressed as a quadratic function of rainfall. Some such relationship is to be expected, for although no moderate variation in temperature could alter the essential processes of soil formation in regions of very low or very high rainfall, it would, however, greatly influence the leaching in areas of moderate rainfall. The coefficients will not, however, be presented and discussed here as they are not derived from sufficiently representative data. Jenny omitted not only the most widely distributed group, the Brown Forest soils, but also left out those parts of the Prairie belt in which the N.S. Quotients and other climatic data deviated widely from the mean. But even the rectification of these points still provides a poor sample of the climates of the principal soil regions. The necessity of using relative humidities to calculate

the N.S. Quotient restricts the choice of centres to first-class meteorological stations among which an undue proportion are either in large cities or on the coast. Until much further progress has been made in the correlation of soils and climate by rigid analysis, it seems advisable to concentrate attention on the simplest climatic data whose distribution is sufficiently well-known to be mapped with an accuracy greatly exceeding that of the soils themselves, and to postpone to a later stage the use of more complex functions involving either other factors or seasonal distributions.

The annual rainfall and temperature were therefore examined on a more representative basis by collecting data from large scale maps at regular intervals of 2° of latitude and longitude. Temperatures were corrected for altitude by means of the factor used in preparing the map of sea-level temperatures. These soil groups were obtained from photographic enlargements of Marbut's maps, but the selection of centres was restricted to the nine soil groups which have been sufficiently studied to have received distinctive names. This gave an area of approaching two million square miles and included the eastern half of U.S.A. and a band across the middle of the western half. The mean values of rainfall and temperature with their standard errors and the regression coefficients of rainfall on temperature within the nine soil groups are given in Table IX in order of increasing rainfall.

Marbut's system of soil classification and maps are well adapted for an examination of the nature of the association of climate and soil properties, for they are based wholly on the features of the soil and not, as in some European systems, on assumed relationships of soil to climate.

The two broadest soil divisions *Pedocals*, in which calcium carbonate, and *Pedalfers*, in which sesquioxides have accumulated, are seen to occur in semi-arid and humid regions respectively. It is obvious too from the standard errors of the mean rainfalls and temperatures that the group of Prairie Soil differs from all others in extending over a very wide temperature range (actually from 3° to 22° C.). There is considerable variation in the relationship of rainfall to temperature within the soil groups. Except for the insignificant values for Grey Desert Soils and Brown Semidesert Soils the correlation is positive and reaches a highly significant value in the large group of Brown Forest Soils. Strangely enough this group was omitted by Jenny from the data previously considered.

In the second part of Table IX the efficiency of the association of the climatic data and soil properties is examined by applying the *z* test to (a) the 9 groups, (b) a class of Marbut's 4 *Pedocals*, (c) a class of Marbut's 5 *Pedalfers*, and (d)



Table IX.—Rainfall and Temperature of Regions of Established Soil Types (Marbut's Soil Classification and Maps).

## (a) Individual Soil Groups.

Soil group.	Number of centres.	Mean values rainfall (R in cm.).	Temperature (T in °C.).	Standard errors of means.		Regression of R on T within groups.	P for equal regression by chance.	R - 3.3 T (leaching factor).
				R.	T.			
<i>Mid-latitude Temperate Pedocals.</i>								
IV-4 Grey .....	13	25	8.5	6.5	3.0	-1.4	>0.1	-3
IV-3 Brown .....	6	33	5.4	6.8	2.5	-2.9	>0.1	15
IV-2 Chestnut coloured .....	5	42	8.3	5.0	1.8	2.3	0.06	14
IV-1 Tchernosem .....	5	60	11.0	11.0	2.4	3.8	0.08	23
<i>Pedalfer.</i>								
V-6 Prairie soils .....	20	87	11.5	13.6	4.8	1.2	0.03	49
V-2 Podzols .....	12	87	5.1	13.3	1.4	5.5	0.05	70
V-3 Brown forest soils .....	32	104	10.1	20.9	2.8	5.2	<0.01	70
V-4 & 5 Red and yellow soils .....	18	127	17.1	13.4	1.8	1.7	>0.1	70
V-8 Ferruginous laterites .....	6	138	20.7	10.7	1.7	2.0	>0.1	69

## (b) Classes of Soil Groups.

	Number of centres.	Efficiency of grouping.			Significant regression coefficients of R on T (for cases in which $P < 0.01$ ).		
		z for rainfall.	z for temperature.	z for $P = 0.01$ .	All centres.	Within groups.	Between groups.
(a) All groups .....	117	2.24	1.74	0.52	4.64	2.36	—
(b) Mid-latitude Pedocal groups .....	29	1.70	1.11	0.77	2.73	—	—
(c) Pedalfer groups .....	88	1.59	1.82	0.64	3.09	—	—
(d) Pedalfer groups (omitting Prairie soils) .....	68	1.50	2.31	0.71	3.54	4.48	3.34

a class of highly leached acid soils obtained by removing the Prairie Soils from the Pedalfer class. In all four classes both rainfall and temperature are significantly associated with the soil groups. As would be expected when all 9 groups are considered together the soils are related more closely to rainfall than that to temperature, i.e., rainfall gives a higher  $z$  than temperature. After eliminating the broad division into semi-arid and humid regions the rainfall remains of greater importance than temperature for the class of Pedocals. Temperature on the other hand becomes the dominant factor (i.e., has the higher  $z$ ) for the Pedalfers, especially when the Prairie Soils group is omitted. It is clear, therefore, that no single combined function of rainfall and temperature can serve as an effective measure of climate over the whole range of soils represented within even the temperate climatic zone and failure of such functions as Lang's and Meyer's is inevitable. The importance of rainfall in the semi-arid region is obvious and the dominance of temperature in the humid belt is doubtless to be explained partly through an effect on evaporation and partly through its influence in determining the type of natural vegetation maintained at any rainfall level. The abnormality of the prairie belt is again shown in the low but significant regression coefficient of rainfall on temperature. This band may justly be regarded as transitional between the humid and the semi-arid regions and doubt may be expressed as to the uniformity of its soils. For its greater part rainfall and temperature are closely related and leaching from their joint action is only slightly below that in the belt of Brown Forest Soils, but in Texas and Oklahoma the temperature rises rapidly without a corresponding increase in rainfall. Geographically the Prairie Soil belt is unique, having common boundaries with four of the eight soil groups listed above and with two broad groups, not yet named by Marbut, lying to the north and south respectively of the Tschernosem belt.

The table of significant regression coefficients illustrates once more the general correlation of rainfall and temperature throughout the greater part of the cultivated area of U.S.A. The division of this regression "between groups" and "within groups" for the four broad classes shows that a significant value "within groups" is obtained only for the class of highly leached soils. It also happens that the mean values of the groups within this class show a highly significant regression of rainfall on temperature which is illustrated in the first part of Table IX by the striking constancy of the values of  $(R - 3.3 T^{\circ} C.)$  for the last four soil groups.

A comparison in Table X of the regression coefficients found for highly leached soils in Table IX with those derived earlier in the paper for the production of constant clays from similar parent materials and for constant

Table X.—Relation of Rainfall to Temperature for Constant Soil Formation.

	Centimetres per ° C.
(1) For constant $\text{SiO}_2/\text{Al}_2\text{O}_3$ in clay.....	4.04
(2) For constant CaO per cent. in clay .....	3.78
(3) For constant drainage in Rothamsted lysimeters .....	3.40
(4) From the distribution of groups of highly-leached soils—	
(a) Means of groups .....	3.34
(b) Within groups .....	4.48

drainage in lysimeters shows fair agreement, suggests that the highly leached groups of soils in the Eastern United States have similar clays. These appear to belong to the halloysite ( $2\text{SiO}_2, 1\text{Al}_2\text{O}_3$ ) type but may contain some excess of sesquioxides. Contrary to expectations it does not seem that the distinction between the principal soil groups in this highly leached class can be made in terms of the alumino-silicates of the clay fraction. It appears rather that the morphological properties used to characterise them depend more on the distribution of iron and organic matter, and that the determining climatic factor is not leaching but temperature. At high temperatures weathering of minerals and decomposition of organic matter proceed rapidly giving deep soils in which iron is precipitated near the surface (Ferruginous Laterites and Red Soils). At low temperatures the iron and organic matter soils are more stable and are leached down to distinct accumulation horizons (Podsols). The Brown Forest soils represent an intermediate class in which movement of colloidal material is impeded by the higher salt and exchangeable base contents maintained by deciduous broad leaved forest covers.

If the factor  $R \text{ cm.} - 3.3 \text{ T}^\circ \text{C.}$  is used as a rough index of drainage and leaching, the values of the rest of the soil groups in Table IX bring out the intermediate position of the Prairies, and low leaching of the semi-arid regions and the absence of drainage in the Grey Desert Soils. The climates of the greater part of U.S.A. may in fact be classified in relation to soil formation by using such a leaching factor in conjunction with the predominance of temperature in the highly leached and of rainfall in the slightly leached regions. As a first approximation the leaching factor may be taken in round numbers as  $(R - 10/3 \text{ T}^\circ \text{C.}) \text{ cm.}$  or  $(R + 24 - \frac{3}{2} \text{ T}^\circ \text{F.}) \text{ inches.}$

*Classification of Climates in Relation to Soil Formation.*

(1) <i>High Leaching</i> .....	Podsol	With increase of tempera- ture. ↓
(leaching factor about 70 cm.)	Brown Forest Soils	
	Red and Yellow Soils	
	Ferruginous Laterites	

- (2) *Transitional* .....

(leaching factor considerably less than 70 cm., rainfall greater than 70 cm.)

Prairie Soils.
- (3) *Low Leaching* .....

(leaching factor and rainfall both less than 70 cm.)

Tschernosem

Chestnut Coloured Soils

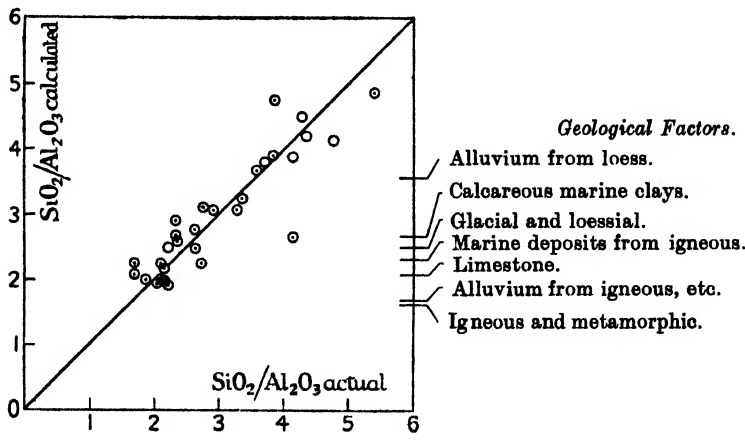
Brown Semi-desert Soils

Grey Desert Soils

↓

With decrease of rainfall

There is some evidence that greater accuracy would be obtained by using a higher regression coefficient of rainfall on temperature for the leaching factor for Podsolised Forest Soils and Brown Forest Soils and a lower factor for Prairie, Tschernosem and Chestnut Coloured Soils. Such expressions would be in harmony with the fact that the forest groups have a greater, and the steppe and prairie groups a smaller, cover of natural vegetation than the average of the centres used in the statistical analysis of both clay composition and the distribution of soil types in this paper. The data available are too scanty to justify closer analysis at the present stage, but the results have demonstrated the desirability of stating at least the simpler climatic data (mean annual



rainfall and temperature) for all centres where fundamental quantitative data on soils are collected. This is especially desirable in the tropics for the simple relationships discussed here appear to break down at higher temperatures and in any case the Ferruginous Laterites of Florida are widely different from the true Laterites. The collection of exact quantitative climatic and soil data with some geological information is particularly necessary in isolated tropical

countries where soil work has only recently been undertaken in order to provide material for a rigid examination of soil forming processes. It should then become possible to develop sounder systems of soil classification than was possible in Europe where too great a concentration on restricted regions, of the use of inadequately established generalisations on the relationships of climate to soils, led to an excessive use of the parent material or the climatic zone respectively, and impeded the discovery of the more fundamental principles in soil formation.

*Summary.*

(1) An attempt is made to separate the effects on soil formation of two quantitative climatic factors and a qualitative geological grouping by means of a statistical analysis capable of application to other geographical and ecological problems. The data discussed consist of chemical analyses of the clay fractions of 30 representative soils and maps of the distribution of the more important soil groups in the United States.

(2) The existence of a high positive correlation between annual rainfall and mean annual temperature in the agricultural areas of the United States accounts for earlier failures to recognise the influence of temperature on clay composition, and also for the essential differences between the nature and distribution of soil types in the United States and in Russia.

(3) The ratio of silica to alumina in the clay fraction is correlated negatively with rainfall and positively with temperature, and the relative effects of rainfall and temperature on clay are closely parallel to their effects on the amounts of drainage through soil in the Rothamsted lysimeter experiments. This suggests that the amount of leaching is the dominant factor in clay formation.

(4) For comparable climatic conditions the lowest  $\text{SiO}_2/\text{Al}_2\text{O}_3$  ratios in the clay fraction are in sedentary soils from igneous rocks, and the highest in the young soils from sediments repeatedly subjected to reworking in water. Highly siliceous clay may be formed in two ways; by weathering in the presence of soluble silicates in the alkaline soils of arid regions or in the immature soils of humid regions where the parent material has been exposed for long periods to the dissolved silica of river waters.

(5) In the regions of highly leached soils in the United States the relationship of rainfall to temperature corresponds to conditions of approximately uniform drainage and clay composition. Within this belt the individual soil groups are more accurately characterised by temperature than by rainfall or leaching. The group of Prairie Soils occurs in a transitional belt between these highly

leached soils and those of semi-arid regions in which rainfall is the dominant climatic factor in the determination of soil type.

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# A FURTHER STUDY OF THE INFLUENCE OF THE PROXIMITY OF A SOLID WALL ON THE CONSISTENCY OF VISCOUS AND PLASTIC MATERIALS

G. W. SCOTT BLAIR\*

## Introduction

In earlier papers,<sup>1</sup> it has been shown experimentally that when a paste of clay or soil in water is forced through a long narrow glass tube, at high shearing stresses where the flow-curves are linear,\*\* and where the rate of shear is not high enough either to necessitate a kinetic energy correction, or to involve turbulence, the following relationship may be applied for certain materials to relate mean velocity of flow ( $v$ ) to shearing stress per unit area at the wall of the tube ( $F$ ).

$$v = \frac{V}{\pi R^2} = \frac{(F-f)R\mu}{4} \quad (1)$$

Where  $V$  is flow per second.

$R$  is radius of tube.

$L$  is length of tube.

$\mu$  is the mobility.

$f$  is the intercept of flow-curve extrapolated to stress axis ( $f$  is zero or positive.)

This is an equation of the type proposed by Bingham,<sup>2</sup> and holds well for certain materials, whereas it has been shown for others that, owing to a modified flow, presumed to take place in the proximity of the walls of the tube, a modification must be introduced into equation (1).

Equation (1) may be rewritten:—

$$\sigma = \frac{dv}{dF} = \frac{R\mu}{4} \quad (1a)$$

For materials showing such modified flow properties, this must again be rewritten:—

$$\sigma - \sigma_0 = \frac{R\mu}{4}$$

Where  $\sigma_0$  is a constant of the material. Since  $\sigma_0$  has rather inconvenient dimensions ( $M^{-1}L^2T$ ), it was also suggested that it might be simpler to extra-

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\*\* It is assumed, as in the earlier paper (I(A) p. 327) that at high rates of shear where the flow-curves are linear, the flow may be regarded as being in general of a "streamline" type; any contribution due of flow of the material as a solid "plug" being neglected. (See also Buckingham (4)).

polate  $\sigma/R$  curves across the  $\sigma$  axis so as to make an intercept,  $(R_0)$ , on the negative  $R$  axis and that the term  $R^3(R + R_0)$  might then be substituted for  $R^4$  in the simple Poiseuille equation.

It was implied that the values for  $\mu$  and  $f$  obtained from the above constructions were those of the bulk of the material; the modified values within the wall-layer were not evaluated. It was shown to be probable that both  $\mu$  and  $f$  are modified in this region. Since  $R_0$  is known to be quite large in many cases, it would seem advisable to derive an expression to account for the flow properties within the modified layer as well.

The idea of a material having more than one value for its plastic constants has been discussed by Hall<sup>3</sup> under the advice of Buckingham, but in this case, the properties varied at different stresses and not at different points in the cross-section of the tube for the same stress at the wall. Buckingham<sup>4</sup> has given an equation to account for slip at the wall, but this refers to slip through a thin water-envelope of constant thickness, so thin that the mean velocity of flow is directly proportional to the stress; and moreover it was shown in a previous paper<sup>1</sup> that this phenomenon is quite distinct from the  $\sigma_0$  phenomenon. An evaluation of an equation of flow is here given for a system in which there is a modified layer of material next the wall of the tube, independent of the radius in thickness, and having a higher mobility, and a lower "yield-value" than those of the bulk of the material.\*

### Evaluation of a Flow Equation

Consider the flow of a paste of the material through a tube of radius  $R$  and length  $L$  such that the mobility of the material is  $\mu$  and the "yield-value" in stress units is  $f$ , except within a layer round the wall having a thickness  $r'$ . The constants within this layer are  $\mu_w$  and  $f_w$  respectively;  $p_0$  and  $p_w$  being used for the pressures corresponding to  $f$  and  $f_w$ . This assumes that there is a hard and fast line of demarkation between the two types of flow, regular and modified. This may or may not be the case: the changes in  $\mu$  and  $f$  at the wall may be gradual, but for the purpose of this discussion a definite value of  $r'$  will be assumed. It is necessary to make some such assumption in order to evaluate the integrals.

The velocity ( $\psi$ ) at any point *within the bulk of the material* is given by:—

$$\psi = \frac{(R^2 - r^2)\mu(P - p_0)}{4L}$$

Where  $r$  is the distance of the point from the axis.

Now the velocity at any point distant  $r$  from the axis, *but within the modified layer*, is given by:—

$$\psi_w = \frac{(R^2 - r^2)\mu_w(P - p_w)}{4L}$$

The total volume of material flowing per second ( $V$ ) is given by the sum of the integrals for each zone, thus:—

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\* "Yield-value". This term is used in Bingham's sense to denote  $f$ .

$$V = \int_0^{(R-r')} 2\pi r \psi \, dr + \int_{(R-r')}^R 2\pi r \psi_w \, dr$$

This becomes, on substituting:—

$$\begin{aligned} V &= \int_0^{(R-r')} \frac{2\pi(R^2r-r^3)\mu(P-p_0)}{4L} \, dr + \int_{(R-r')}^R \frac{2\pi(R^2r-r^3)\mu_w(P-p_w)}{4L} \, dr. \\ &= \frac{\mu\pi(P-p_0)}{2L} \int_0^{(R-r')} r(R^2-r^2) \, dr + \frac{\pi\mu_w(P-p_w)}{2L} \int_{(R-r')}^R r(R^2-r^2) \, dr. \end{aligned}$$

This gives:—

$$V = \frac{\pi\mu(P-p_0)}{8L} (R^4 - 4R^2r'^2 + 4Rr'^3 + r'^4) + \frac{\pi\mu_w(P-p_w)}{8L} (4R^2r'^2 - 4Rr'^3 + r'^4)$$

Setting  $v$ , (mean velocity)  $= V/\pi R^2$ .

and  $F = PR/2L$ ;  $f = p_0R/2L$ ;  $f_w = p_wR/2L$ .

we get:—

$$V = \frac{FR}{4} (\mu\alpha + \mu_w\beta) - \frac{fR}{4} (\mu\alpha + \mu_w\beta)$$

where

$$\alpha = 1 - \frac{4r'^2}{R^2} + \frac{4r'^3}{R^3} + \frac{r'^4}{R^4}$$

and

$$\beta = \frac{4r'^2}{R^2} - \frac{4r'^3}{R^3} + \frac{r'^4}{R^4}$$

Now set  $\frac{dv}{dF} = \sigma$

$$\sigma = \frac{R}{4} (\mu\alpha + \mu_w\beta) \quad (3)$$

In the treatment given in the previous paper, we had, as in the equation

(2) above,  $\sigma - \sigma_0 = \frac{R\mu}{4}$

$$\text{or } \sigma_0 = \sigma - \frac{R\mu}{4}$$

Hence  $\sigma_0$  is really given by:—

$$\begin{aligned} \sigma_0 &= \frac{R}{4} (\mu\alpha + \mu_w\beta) - \frac{R\mu}{4} \\ &= \frac{R[\mu(\alpha - 1) + \mu_w\beta]}{4} \end{aligned}$$

and  $R_0 = -\frac{4\sigma_0}{\mu}$  (setting  $\sigma = 0$  in the equation above.)

$$= -\frac{R[\mu(\alpha - 1) + \mu_w\beta]}{\mu}$$

(The sign is negative because  $R_0$  is an intercept on the negative  $R$  axis.)

In order to solve these equations, it is necessary to know something about either  $r'$  or  $\mu_w$  and  $f_w$ .

At present, experimental data are not forthcoming, but the above treatment stands as a theoretical prediction, in the hope that it will some day be verified, amplified, or rejected, in the light of future experiment.

### Summary

The case of a material streaming through a tube and having a layer showing modified consistency constants in the immediate neighbourhood of the wall of the tube is discussed, and the empirical "correction" constants proposed in the previous paper are expressed in terms of the consistency constants, (modified and otherwise,) of the material.

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*April 19, 1930.*

# THE INFLUENCE OF THE PROXIMITY OF A SOLID WALL ON THE CONSISTENCY OF VISCOUS AND PLASTIC MATERIALS. III

BY R. K. SCHOFIELD AND G. W. SCOTT BLAIR\*

It has been recognized for some time that, if a paste of clay or soil in water is forced through a narrow tube, there are certain limits of pressure between which the flow is telescopic. Within this range the rate of increase of extrusion with increase of pressure is constant within the present limits of experimental accuracy. Except in the case of very thin pastes the straight line obtained by plotting the volume  $V$  extruded in unit time against the pressure  $P$  does not on extrapolation pass through the origin, but makes a positive intercept on the pressure axis. Nevertheless, according to the treatment of Bingham<sup>1</sup> as amended by Buckingham<sup>2</sup> and independently by Reiner<sup>3</sup> the slope of this line should give the mobility of the material, a constant of which the reciprocal is closely analogous to the viscosity of a true fluid and has therefore been called the pseudo-viscosity.

In an investigation described in the first paper of this series<sup>4</sup> it was found that with many pastes the value obtained for the mobility from Bingham's equation depended upon the diameter of the tube used. It was shown that this result could only be accounted for by supposing a layer of material near the wall of the tube to have a mobility different from that of the bulk of the paste passing through the centre of the tube. It appeared sufficient for the purpose of obtaining this true mobility to assume that the thickness of the modified layer is small in comparison with the radius of the tube. Since the discrepancies are often considerable, it is desirable to obtain assurance on this point, or failing this, to form an estimate of the error that may be introduced in cases where the modified layer forms an appreciable fraction of the radius of the tube.

An attack was made on this in a second paper.<sup>5</sup> The method used, though direct, was rather cumbersome and was not quite complete.\*\* The following treatment is more concise and enables the implications to be more clearly set out.

Imagine that a material of mobility  $\mu_\omega$  is flowing through a tube of radius  $R$ , and that the line  $ACB$  in Fig. 1 represents, by the distance above  $AB$ , the velocity distribution within the tube. This curve is of course a section of a surface which can be traced within the tube, and the volume enclosed between this surface and the cross-section at  $AB$  is a measure of the volume extruded in unit time. If now it be supposed that, instead of a constant mobility across

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\*\*In giving an expression for the velocity of a point within the bulk of the material, the velocity given was that relative to the boundary distant  $\delta$  from the wall (the symbol  $r'$  was used in the earlier paper). There should also have been a term in addition to allow for the velocity of the boundary relative to the wall of the tube.

the tube, the value  $\mu_\omega$  only holds between the wall and an imaginary cylindrical boundary distant  $\delta$  from it, and that within this boundary the mobility is  $\mu_0$ , the surface within the boundary will have a section represented by the line ADB. The total extrusion will, in this case, be less than that in the first case by an amount given by the volume enclosed between the two curved surfaces. This volume may be taken to represent an extrusion through a tube of radius  $(R - \delta)$ .

Within the pressure range over which the flow curves are straight, its slope  $dV/dP$  has, for the case where the mobility is  $\mu_\omega$  over the whole cross-section, a value

$$dV/dP = \frac{\pi R^4}{8L} \mu_\omega$$

$L$  being the length of the tube which is long in comparison with its radius  $R$ .

To obtain the slope of the flow curve in the second case an amount must be subtracted equal to the rate of change with  $P$  of the volume enclosed between the two curved surfaces of Fig. 1. This is

$$\frac{\pi(R - \delta)^4}{8L} (\mu_\omega - \mu_0)$$

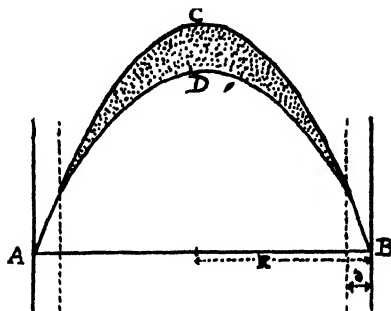


FIG. 1

Thus in the second case

$$dV/dP = \frac{\pi R^4}{8L} \mu_\omega - \frac{\pi(R - \delta)^4}{8L} (\mu_\omega - \mu_0)$$

The slope  $\sigma$  of the graph of mean velocity of extrusion  $(V/\pi R^2)$  against stress on the wall of the tube  $(PR/2L)$ , used in the earlier papers, is readily obtained by multiplying both sides by  $2L/\pi R^3$

$$\text{thus } \sigma = \frac{d(V/\pi R^2)}{d(PR/2L)} = \frac{2L}{\pi R^3} \cdot \frac{dV}{dP} = \frac{1}{2} R \mu_\omega - \frac{1}{2} \frac{(R - \delta)^4}{R^3} (\mu_\omega - \mu_0)$$

If now we suppose that the change from  $\mu_\omega$  to  $\mu_0$  does not take place abruptly at a fixed distance  $\delta$ , but that the mobility at a point in the tube is a function of its distance  $\delta$  from the wall (but not of  $R$ ), the expression for  $\sigma$  becomes

$$\sigma = \frac{1}{2} R \mu_\omega - \frac{1}{2} \int_{\mu_\omega}^{\mu_0} \frac{(R - \delta)^4}{R^3} d\mu$$

provided that  $\mu$  reaches the constant value  $\mu_0$  before  $\delta$  reaches  $R$ . On expansion this becomes

$$\sigma = \frac{1}{2} R \mu_\omega - \frac{1}{2} \int_{\mu_\omega}^{\mu_0} R d\mu + \int_{\mu_\omega}^{\mu_0} \delta d\mu - \frac{3}{2} \int_{\mu_\omega}^{\mu_0} \frac{\delta^2}{R} d\mu + \text{etc.}$$

$$= \frac{1}{4} R \mu_0 + \int_{\mu_0}^{\mu_0} \delta d\mu - \frac{3}{2R} \int_{\mu_0}^{\mu_0} \delta^2 d\mu + \text{etc.}$$

According to this expression the graph  $\sigma$  against  $R$  should be of the type shown by the full line in Fig. 2. At high values of  $R$ , the curve approaches an asymptote which has a slope  $\frac{1}{4}\mu_0$  and which is represented by the broken line. The departure of the actual curve from this asymptote depends on the relative magnitudes of  $R$  and  $\bar{\delta} = \int \delta^2 d\mu / \int \delta d\mu$ . Although no sharp boundary has been assumed to exist between the bulk of the material and the

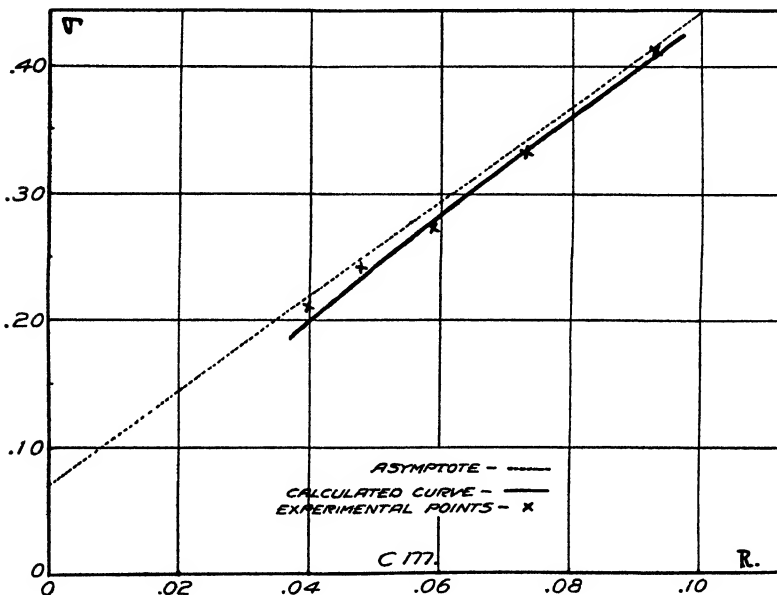


FIG. 2

modified layer, nevertheless  $\bar{\delta}$  is a kind of effective thickness of the modified layer near the wall. The asymptote was drawn in by inspection to give a convenient intercept on the  $\sigma$  axis. The position of the curve was then calculated assuming a value for  $\bar{\delta}$  equal to  $8 \times 10^{-3}$  cm. (being 20 per cent of the radius of the smallest tube used in the experimental work). The experimental points are for the Broadbalk field subsoil for which the full data were given in the first paper. It is evident that although a straight line could fairly be drawn through the points, as was done in the earlier paper, the experimental uncertainty is such that the possibility of a slight curvature is not ruled out. Actually a number of curves could be drawn each of which would pass sufficiently near the points so that the position of the asymptote is to some extent arbitrary.

### Conclusions

An examination of all our data shows that no reliable estimate can be made of the curvature of the  $\sigma - R$  graphs and thus no evaluation of  $\bar{\delta}$  is yet possible. At the same time it is apparent that a value of  $\bar{\delta}$  amounting to  $8 \times 10^{-3}$  cm. could escape detection owing to present experimental uncertainties.

Were it possible to draw the true asymptote, its intercept on the  $\sigma$  axis

would, according to the forgoing analysis, give the value of  $\int_{\mu_0}^{\mu_0} \delta d\mu$ . The

intercept made by the best straight line through the points, which we have called  $\sigma_0$ , is evidently somewhat less than this.

With regard to the value of  $\mu_0$ , the mobility of the material in bulk, we may conclude that although a determination based on the slope of the best straight line drawn through experimental points on the  $\sigma - R$  diagram is more accurate than any value calculated by the Bingham formula for a single tube, the value may still be a little too low owing to a finite thickness of the modified layer.

### Summary

Evidence was obtained in an earlier paper that, when a clay or soil paste is forced through a narrow tube, a layer of material next to the wall has a consistency different from that of the bulk of the material. It was first assumed, for simplicity of treatment, that the modified layer is very thin compared with the radius of the tube; but later an attempt was made to remove this restriction.

A simpler and more complete treatment is now given, and it is concluded that a solid wall may modify the consistency of the material at an appreciable distance from it, but that the accuracy of the available data is insufficient to enable any reliable estimate to be made of the thickness of the modified layer. It is shown that, even when its effective thickness is as much as 20 per cent of the radius of the tube, the construction proposed in the first paper for determining the mobility of the material in bulk gives a close approximation to the true value. A full application of the extended treatment must await the development of more accurate experimental methods.

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# STUDIES IN SOIL CULTIVATION.

## V. ROTARY CULTIVATION.

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(With Four Text-figures.)

### INTRODUCTION.

WHEN steam power was first applied on an extensive scale to cultivation operations in the first half of the last century, its main purpose was to haul the traditional cultivation implements, which were built to a larger size for this form of power. Multi-furrow ploughs, deep subsoilers and powerful cultivators were evolved, and the designs rapidly settled down to the present-day form of the familiar steam cultivation tackle.

However, a number of people considered that full use was not being made of this new form of power. Its essential characteristic was that it supplied a rotary motion, and it appeared to them wasteful, and even incorrect, to convert this rotation to a translatory motion for hauling the variety of implements necessary to produce a seed-bed. The identical result should be obtained—in theory at any rate—in one operation instead of several, by a transverse horizontal shaft fitted with radial tines, and revolved by power from the same steam engine that drew it across the field. The rotating tines would comminute and thoroughly mix the soil, leaving a seed-bed behind them. This process is now generally known as rotary cultivation. It was very persuasively described by Hoskyns in his *Talpa, or The Chronicles of a Clay Farm*, one of the most delightful books in the literature of agriculture:

Is it not astonishing, with such experiences as we have before us in England, that since the first introduction of Steam-power to the notice and assistance of mankind, nobody has ever yet attempted to apply it *in its own way* to the definite and simple work of cultivation? It is put to cut chaff, to make saw-dust, to granulate powder, to make pins' heads, to reduce all sorts of *coarse material into fine*—and all by wheels,—*circular motion*, and nothing else, for nothing else will it accept,—but nobody can persuade his mind to believe that by the self-same action, and no other, it can cut

<sup>1</sup> Eleven past and present members of the Rothamsted staff and four voluntary workers have been associated with these experiments during the period 1926-9: W. B. Haines, E. M. Crowther, J. H. Coutts, C. Heigham, G. H. Cashen, G. W. Scott Blair, R. K. Schofield, H. G. Miller, A. R. Clapham, E. H. Gregory, J. Wishart; K. T. Hartley, D. W. Baker, G. E. Blackman, M. A. Sabet.

up a seam of soil eight inches deep and five feet wide, and leave it behind granulated to as coarse or fine a texture as the nature of the seed or season may require, and inverted in its bed. It is not ploughing, it is not digging, it is not harrowing, raking, hoeing, rolling, scarifying, clod-crushing, scuffling, grubbing, ridging, casting, gathering, that we want: all these are the time-honoured, time-bothered *means* to a certain RESULT. That result is—a seed-bed: and a seed-bed is, simply described, a layer of soil from six to twelve inches in depth, rendered fine by comminution, and as far as possible inverted during the process.

Machines embodying this principle were tried but without much success. They were heavy and cumbersome and did not produce a satisfactory tilth. On the other hand, a fair measure of success attended certain machines designed to produce not a seed-bed but a rough cultivation analogous to that given by the ordinary cultivator. These implements were “diggers” rather than rotary cultivators, the action of the spuds fixed to the rotating tines being to break the soil into large lumps and more or less to invert them. A few machines of this type were in use on heavy land in Essex until recently.

It appeared, therefore, that this pioneering work on rotary cultivation failed for two main reasons: firstly, the weight of the cumbrous machine, and secondly, the inability of the tines to produce as satisfactory a tilth as that obtainable with the traditional implements.

The invention of the internal combustion engine removed the first of these objections: the modern type is of considerably less weight per horse power than the old steam engine. The second objection is naturally more serious. Recent work on soil has emphasised the complicated nature of soil tilth and its controlling factors. But there is no *a priori* reason to assume that we have reached finality, either in the design of implements or in our cultivation methods.

These considerations led to the decision to make comparisons of rotary and traditional cultivation over a series of years at Rothamsted under field conditions. Experiments were made during the years 1926–9 inclusive, and the results form the subject of the present paper.

#### DESCRIPTION OF ROTARY CULTIVATION MACHINE.

The machine used for the Rothamsted experiments is of a type that has already been introduced for cultivation work in orchards, market gardens and commercial glasshouses.

It consists essentially of a 5 H.P. engine mounted on and geared to the two land-wheels, with a transverse horizontal shaft at the back, also driven by the engine. This milling shaft carries twelve radial tines, six on each side of the central line of the machine, and disposed symmetrically

about the shaft. The machine is steered by a long pair of handles which also carry the controls.

There are two forward speeds, about 2 and  $\frac{3}{4}$  miles per hour. The rotation speed of the milling shaft is about 150 R.P.M., so the extent of forward movement for one complete revolution of the miller is about 14 in. at the high speed and 5 in. at the low speed. During this unit of forward movement each of the tines makes a complete revolution down into and up out of the soil. Each tine point, which is at a distance of about 8.5 in. from the axis of the milling shaft, therefore traces out a cyclic curve, the form of which for the fastest and slowest speeds is shown in Fig. 1. The part of this curve which is executed in the soil depends on the depth of tillage, which is adjusted within the limits of 2 in. and 10 in. by a depth shoe underneath the miller gear-box. A scale of depth is also shown in Fig. 1. Reference to the curve for the fastest speed shows that, as far as any single tine is concerned, a certain amount of the soil is left unstirred: this is represented by the area between the two loops of the cyclic curve, and has a minimum length of  $3\frac{1}{2}$  in. and a maximum length of 14 in., this latter being represented by the distance between the lowest points of successive loops. But, as the tines are arranged in pairs roughly at opposite ends of a given diameter, the unstirred area in the figure is effectively dealt with by the second tine; the cyclic loops for this latter fall midway between those shown in the figure. In actual practice the tines are "staggered" with respect to the common diameter, so that they do not revolve in the same vertical plane in order to give a more uniform distribution of the cultivating action over the width of the work. It is evident that, in this case, some of the soil is only indirectly stirred by the tines<sup>1</sup>: the tine point will break away a lump of soil, and this may be carried round with the tine and thrown upwards against the miller hood, or it may be forced to one side and come under the action of an adjacent tine. On the other hand, the soil in the direct path of each tine is undoubtedly well pulverised, since in addition to the tine point, the whole body of the tine itself exercises a disintegrating action on the soil. Normally, therefore, for soil not initially in good physical condition (in which case almost any form of cultivation will produce a good tilth), the tilth produced by rotary cultivation will be a mixture of coarse and fine particles. This is an important point, because it is often urged that rotary cultivation produces too fine a tilth. The results discussed below will show this is not necessarily the case. Naturally

<sup>1</sup> When broad scuffling tines are fitted for shallow cultivation a greater bulk of the soil is directly stirred.

when the slowest forward speed is used much more stirring of the soil is effected. This is brought out by comparing the two curves in Fig. 1.

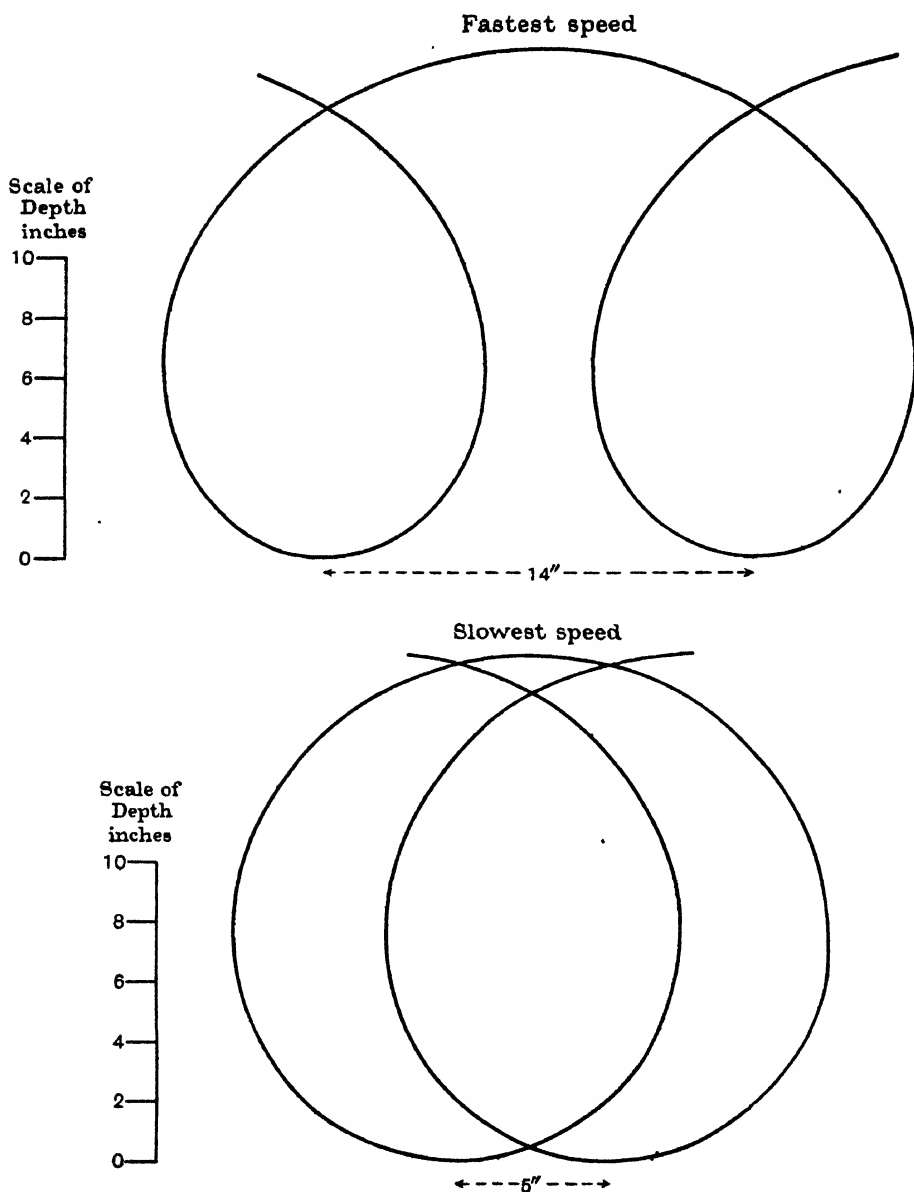


Fig. 1. Path of the tine points for fastest and slowest forward speeds.

## PLAN OF THE ROTHAMSTED EXPERIMENTS.

The bulk of practical experience of rotary cultivation has so far been secured on light and sandy soils. The Rothamsted experiments provided a severer test owing to the nature of the soil. It is a heavy loam containing numerous flints, and demands some care in cultivation. For these reasons it was decided to confine the first series of experiments to spring cultivation and spring-sown crops only, and to defer the studies on autumn cultivations and autumn-sown crops to a later period. This second series of experiments is now in hand.

In all cases, therefore, unless stated to the contrary, the comparative experiments between rotary and ordinary cultivation were made on land that had been ploughed in the autumn or winter in the usual way. It will be realised that restriction of the experiments to spring cultivations necessarily postpones an answer to the question whether rotary cultivation is a satisfactory substitute for all our present cultivation operations, but it seemed advisable to attack the more difficult question of autumn cultivation only after some experience had been gained.

The observations fall into two main sections: measurement of the crop yield and records of germination and plant growth; measurement with a series of sieves of the amount of soil pulverisation or comminution produced by the given cultivation implement, and records of soil moisture and temperature at intervals during plant growth.

These two sections will be discussed separately below.

## SECTION I. THE CROP RESULTS 1926-29.

*The 1926 experiments.* This was made on Sawyer's field, and was a comparison of three different methods of preparing a seed-bed for swedes. The preparatory "autumn" ploughing of 1925 was unavoidably delayed until February 1926. The land was wet and it was difficult to bury weeds. The land was left in the furrow until May 19th, when the triplicate plots received the following cultivations given on the stale furrows in the manner stated:

Series S. Rotary cultivated, rolled, drilled, rolled.

Series F. Tractor cultivated, harrowed, drilled, harrowed and rolled.

Series N. Ridged with bouting plough, rolled, drilled, rolled.

On each plot the depth of cultivation was 6 in.

As the object of the experiment was to study the effects of cultivation differences, it was considered advisable not to apply farmyard manure, which might have had the effect of masking these differences. A dressing



of fish meal, 4 cwt. p.a., superphosphate, 3 cwt. p.a., and muriate of potash, 1 cwt. p.a., was applied about a week before the experiment began, and a top-dressing of nitrate of soda, 1 cwt. p.a., was also given after singling.

In spite of the delay in winter ploughing and the weedy nature of the ground, the soil itself was in excellent condition, due no doubt to the severe frosts of the winter. Each of the three methods produced a satisfactory tilth. The rotary cultivation plots, however, were noticeably different from the other two treatments; the tilth was much looser and the foot sank some distance into it. This result is readily understandable. The rotating tines are travelling in an upward direction as they are leaving the soil. Not only is the tilth thoroughly loosened by this action, but a certain amount of the soil is actually thrown upwards, to fall more or less gently on the soil surface. It appeared that it would be difficult to use a horse drill on the soil in this condition, so a light rolling was first given to consolidate the soil.

After sowing, the plots were kept under observation. It was noteworthy that germination occurred earliest on the rotary cultivated plots, and this initial advantage was more than maintained in the early stages of growth. Shortly after germination the young plants were attacked by fly. The attack was not severe except on the ridged seed-bed plants, where a certain amount of re-seeding was necessary; the rotary cultivation plots escaped with little damage.

Frequent observation of the rate of growth was made after germination. Without exception the rotary cultivation plots were superior, the differences between the plants on these plots and the others being sufficiently great to be apparent to the naked eye. In view of this, and also because of the uncertainties due to the re-seeding of part of the Series N. plots, no detailed measurements of plant growth were taken.

All the plots suffered somewhat from weeds, and the available labour for horse hoeing, which was done at intervals during July and August, was insufficient to keep the weeds under. Docks and thistles were worst on the rotary cultivation plots, but this cannot be ascribed with certainty to the action of the rotating tines in breaking into smaller pieces the viable weed roots present at the time of cultivation. Although this effect undoubtedly occurred, it was also observed that many roots were pulled to the surface, where they died.

Singling was done unduly late in the season—again owing to labour difficulties and the large acreage of roots on the farm. To meet this situation the roots were first “bunched” by drawing across the rows a

cultivator frame carrying large ducksfoot tines grouped at suitable distances, so that hand labour was confined to singling the bunched roots. This operation left more roots on the rotary cultivation plots than on the remainder. In the first place, there were initially more gaps on the latter plots, owing partly to the inferior germination, and partly to the attack of fly already mentioned. In the second place, a number of the plants on the rotary plots either re-established themselves, or resisted the tines of the cultivator sufficiently to remain established in spite of injury. A similar observation was made on a closely adjoining area on which this method of bunching had to be employed (1), and a significant correlation (+ 0.671) was subsequently obtained between the ploughing draught (2) and the number of roots at harvest; the higher the draught the more resistant the soil and, therefore, the greater tendency both for the cultivator to ride out of its work and for partially uprooted plants to re-establish themselves.

This effect in the present investigation was closely associated with a field observation now to be described, which was both striking and unexpected. The loose tilth produced by the rotary cultivation and its effect on germination and plant growth have already been mentioned; it was observed that, in common with the other plots, the tilth was becoming more consolidated as time went on. This was of course to be expected; the Rothamsted soil tends to "cap" or to form a hard crust about 1 inch thick, and surface cultivation to break this crust is a standard procedure. On the rotary cultivation area, however, the effect was far more intense, and these plots could be picked out by the mere feel of the land underfoot; the hardening or capping effect had extended to a much greater depth in this case. The effect on the plants was equally striking. Before the capping they were obviously ahead of those on the other plots, but afterwards they made little further growth, and from being the best and most forward plants, they finished up the season as definitely the worst and least developed. The harvest yields are given in Table I.

The yields on the S. plots are significantly below either of the other two treatments, which do not differ significantly from each other. This difference is all the more striking in view of the significantly greater number of roots on the S. series of plots as shown in the table.

The negative factor responsible for the differences in root number was the severer losses due to attack of fly on the N. and F. series, only partly remedied by the re-seeding, while the positive factor was the survival of many roots on the S. series, owing to the ineffective method of "bunching" with the cultivator on the already hardened soil. A

measure of the size and weight distribution of the roots was obtained from some 230 sample roots from each plot. These were obtained by pacing consecutively up one row and down the next, and pulling the nearest root after a definite number of paces. The size distribution was measured on the basis of bulb diameter, using a set of ten gauges, each of which was nine-tenths the size of the preceding one. The gauges began at 17.3 cm. and did not extend below 7.8 cm. in diameter. All roots 7.8 cm. in diameter were put into the bottom class, which may be regarded as representing the number of useless roots for practical purposes. The results are shown in Fig. 2, which demonstrates clearly that the average size of the roots is lowest on the rotary cultivated plots and greatest on the ridged plots. The size distribution curve for each series also shows a considerable number of roots in class O, *i.e.* of less diameter than 7.8 cm., especially on the rotary cultivation series, due to the survival of many plants from the bunching operation and the severe check to growth following the extensive hardening of the soil.

Table I. *Weight of roots per acre in tons, and number of roots per acre (1926).*

Plot No.	Rotary cultivation (S.)	Flat seed-bed (F.)	Ridged seed-bed (N.)
1	9.88	12.25	11.56
2	9.55	10.84	12.86
3	8.77	10.06	11.00
Average	9.40	11.05	11.81
Average percentage of general mean	87.5	102.8	109.8
Average no. of roots p.a.	15,784	13,157	10,693

Standard error of averages: 0.403 ton and 3.75%; 821.7.

Significant difference: 1.140 tons and 14.4 %; 2324.

The weight distribution for the sample roots was taken to the nearest ounce for each root, and in Fig. 3 the results are given in 5 oz. steps. As would be expected, these curves are more regular than those of Fig. 2, and they bring out very clearly the differences in growth with the three cultivation treatments.

The 1926 experiments, then, showed a distinct advantage for rotary cultivation in the germination of seed and the early stages of growth, followed by a remarkable reversal in the later stages due to extensive hardening of the tilth, resulting in an appreciable reduction of yield. This effect at once raised the question whether it was an inevitable accompaniment of this form of cultivation on heavy soil, and to what extent it could be prevented.

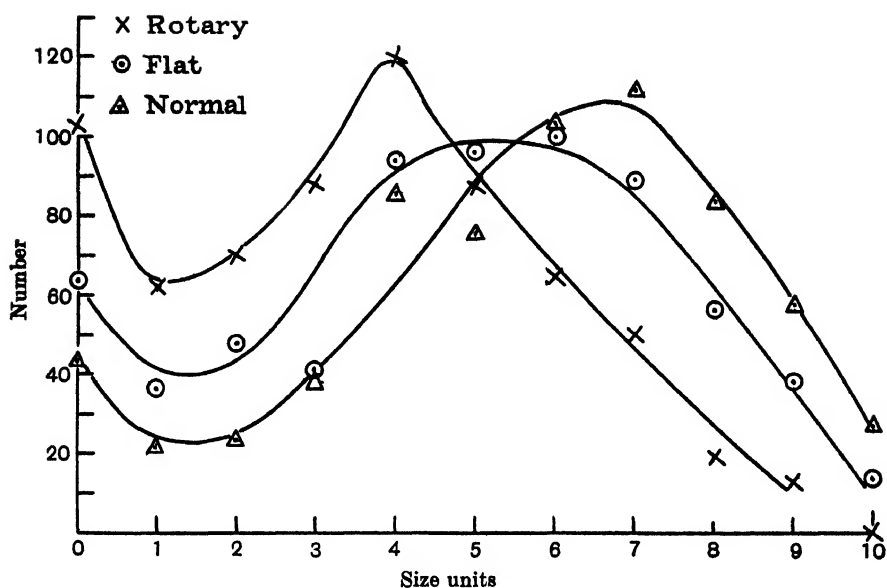


Fig. 2. Size distribution of roots, 1926 experiments.

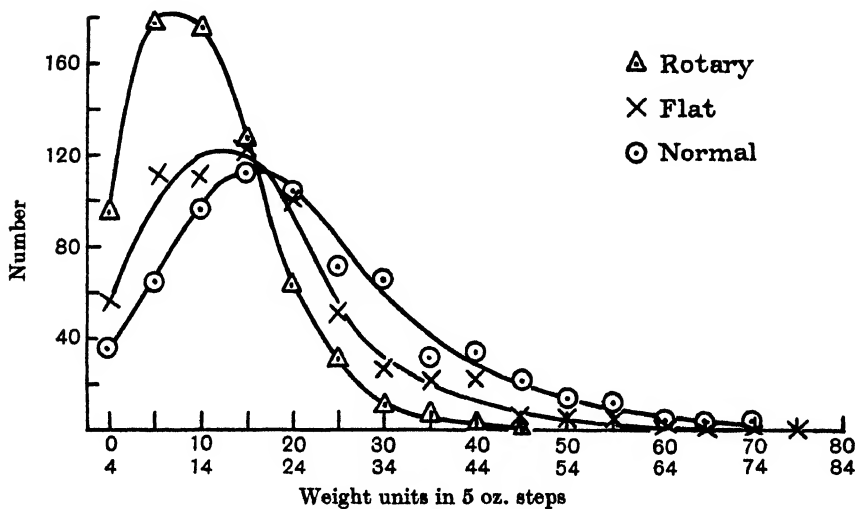


Fig. 3. Weight distribution of roots, 1926 experiments.

*The 1927 experiments.* It was decided to follow up the 1926 results by retaining the same plots, and to sow them to another spring crop. Barley was selected as the normal crop in the rotation. As the comparative experiments were to be confined to spring cultivations only, and in view of the very hard condition of the soil on the S. series, the whole area was cross-ploughed in January. Unfavourable weather prevented further operations until April.

It was proposed to repeat the rotary cultivation on the S. plots of 1926, to horse cultivate and disc harrow the F. series, and to plough (lengthwise), drag harrow and ring roll the N. series. Thus, as in 1926, rotary cultivation would be compared with two other standard traditional methods of spring cultivation. Owing to a mechanical defect, only one of the three rotary cultivation plots received this treatment; the remaining two were treated in the same manner as the F. series. The treatments in 1927 compared with those for 1926 are given below for convenience of reference.

*All plots ploughed.*

1926			
Spring treatment	Rotary cultivated, rolled, drilled rolled	Tractor cultivated, harrowed, drilled, harrowed, rolled	Ridged with bouting plough, rolled, drilled, rolled
Descriptive letter	S.	F.	N.

*All plots cross-ploughed.*

1927			
Spring treatment	(One plot only) ro- tary cultivated, harrowed, drilled, harrowed & rolled, (other two plots) treatment as in C.	Horse cultivated, disc harrowed, harrowed, drilled, harrowed & rolled	Ploughed lengthwise, drag harrowed (twice), ring rolled, harrowed, drilled, harrowed & rolled
Descriptive letter	S.' for single plot. C.' for other two.	C.	P.

The operations were spread over the period April 5th–May 9th, and included manuring as follows: superphosphate, 3 cwt. p.a., muriate of potash, 1 cwt. p.a., and sulphate of ammonia, 1 cwt. p.a. The wet weather in the early part of the year and a dry spell previous to the spring cultivations produced an unkindly condition in the soil, and made it difficult to obtain a tilth. In consequence, sowing was delayed and the harvest was correspondingly late.

Observations of the plots were made at intervals, and showed unmistakably that growth on the old S. series of 1926 (*i.e.* the S.' plot and the two C.' plots in the present series) was much better than on any of the other plots. There was no sign on the one rotary cultivated plot (S.') of

the remarkable effect obtained with swedes in 1926. The superiority of the S.' and C.' plots over the remaining six was easily visible to the naked eye, and was carried on into the harvest yields both of grain and straw. The mean results are shown in Table II.

Table II. *Yields per acre. (Barley 1927.)*

Plots	...	...	...	S.' & C.'	C.	P.
Grain (bushels)				27.8	21.6	21.9
Percentage of general mean				117.0	91.0	92.0
Standard error 1.16 bush.				Significant difference 3.28 bush.		
Straw (cwt.)				21.7	18.6	18.3
Percentage of general mean				111.1	95.3	93.6
Standard error 0.46 cwt.				Significant difference 1.30 cwt.		

This table shows that the plots which, in 1926, gave yields significantly below the remainder, have in 1927 given yields significantly above the others. As there was no appreciable difference in yield between the S.' and the two C.' plots in spite of the difference in cultivation, the effect must be ascribed to some residual action from the preceding year. There is the obvious possibility that more plant food was available for the barley on the old rotary cultivation plots owing to the lower yield of swedes given in the preceding year. But a simple manurial residual effect of this nature seems very unlikely in view of the small amounts of nutrients involved. There was a deficiency of approximately 2 tons of swedes on the S. series of 1926, and an excess of approximately 6 bushels of barley, and the corresponding amount of straw, on the same plots in 1927. Taking accepted figures for the N, P and K contents of these crops, we obtain:

Crop	Lb. per acre.		
	Nitrogen	Potash	Phosphoric acid
Swedes (2 tons)	10.0	9.0	2.4
Barley (6 bushels & straw)	7.2	5.4	3.1

There is admittedly an approximate equality in the two sets of figures, but the amounts are small in comparison with the manures that were given to the barley crop; further, a proportion of the nutrients would have been washed out from the soil during the winter. The effect is by no means easy to understand, and, as something very similar also appeared in the later experiments of 1927 and 1928, described below, it appears to be a probable accompaniment of rotary cultivation.

*The 1928 experiments.* These experiments were transferred from Sawyer's field to Great Harpenden field, to allow of a more extended series of plots.

A composite soil sample was taken for mechanical analysis from the experimental area of each field. The figures show that the areas were practically identical in mechanical composition, and direct comparison of the experimental results is therefore legitimate.

*Mechanical analysis (Revised Official Method A.E.A. (3)).*

	Coarse sand	Fine sand	Salt	Clay	Air dry mois- ture	Loss by solu- tion	CaCO <sub>3</sub>	Differ- ence	Total
Sawyers	12.8	40.2	23.0	19.5	2.2	0.7	—	1.6	100.0
Gt. Harpenden	7.8	41.3	23.3	22.3	2.6	0.8	—	1.9	100.0

Swedes were selected for the crop, and the experiment included trials of possible methods of preventing the hardening of the soil noted with rotary cultivation in 1926, with the idea that the superiority in the early stages of growth might then be maintained over the whole growth period.

The land was winter ploughed in December 1927, and left in the furrow until May 4th, when a thistle cutter was used to kill surface weeds, which were fairly numerous. On May 5th the following manure was sown: sulphate of ammonia 2 cwt., superphosphate 2 cwt., and muriate of potash, 2 cwt. (all per acre).<sup>1</sup>

The following four cultivation treatments, each in quadruplicate, were given over the period May 7th–9th:

Series A. Horse ploughed, harrowed (twice) and ridged.

Series B. Rotary cultivation followed by ridging.

Series C. Rotary cultivation (left flat).

Series D. Rotary cultivation (left flat) to be followed by a second shallow rotary cultivation at a later date.

Series A. and C. were a repetition of the 1926 trials; Series B. and D. were intended to compare two possible methods of overcoming the hardening of the soil. Method D. was based on the idea that if the surface "cap" were broken at an early stage it might not extend downwards; method B. embodied the assumption that the "cap" would be less likely to form on the sloping surface of the ridges than on the level soil.<sup>2</sup>

<sup>1</sup> A portion at one end of the experimental area, covering nearly the whole of the first block, inadvertently received a dressing of dung which was being applied over the rest of the field. Statistical examination showed that the response to the cultivation treatments was very much the same on the dunged and undunged areas, while the extra variation due to the higher yield of the dunged plots, being mostly a block effect, was eliminated from the comparisons. The yield data given in Table III were not, therefore, corrected for this effect.

<sup>2</sup> The ridging would normally be done at the same time as the rotary cultivation, by a ridging attachment fitted behind the rotating shaft. In this experiment suitable ridging breasts were not available, and an ordinary bouting plough (horse drawn) was used.

Seed was sown on May 9th, and the whole area was then rolled. Germination was again earliest on the rotary cultivation plots, but there were no marked differences in subsequent growth. Singling was done on June 18th, and was immediately preceded by hoeing on all the plots. A second horse hoeing was given on July 3rd, and a final hand hoeing on July 12th. Although these hoeings interfered with the original scheme, in that they would probably tend to prevent the soil-hardening effect which treatments B. and D. were designed to study, it was judged essential that they should be carried out in view of the weed infestation. Careful watch kept on the whole area from the date of germination disclosed no signs of capping, or of differences in growth between the various cultivation treatments. It was reasonable to conclude either that the capping effect was absent altogether, or that any tendency for it to occur could be defeated by a light cultivation in its early stages. At the time of the last hoeing on July 12th, the plants had made considerable growth; it was evident that the second light rotary cultivation arranged in treatment D. would involve some danger of damage to the leaves of the plants from the rotating tines. Although there was still no sign of capping, it was decided to give the second rotary cultivation to meet the possible contingency of the effect occurring later in the season. The operation was done on July 20th, and a certain amount of damage to the leaves of the plant was unavoidable.

The roots were counted, lifted, and weighed during the period November 21st–25th, and a series of girth measurements were taken on sample roots from each treatment. The yields are summarised in Table III, and the frequency curves for growth measurements are shown in Fig. 4.

Table III. *Yields and root numbers. (Swedes, 1928.)*<sup>\*</sup>

Series	A.	B.	C.	D.		
				As C. with		
Cultivation treatment	Ridged	Rotary cultivation and ridged	Rotary cultivation left flat	second rotary cultivation	Means	Standard errors
<b>Roots:</b>						
Tons per acre	22.67	22.80	20.12	18.02	20.90	0.50
Percentage	108.5	109.1	96.2	86.2	100.0	2.39
<b>Tops:</b>						
Cwt. per acre	21.03	20.92	17.08	16.05	18.77	1.13
Percentage	112.0	111.4	91.0	85.5	100.0	6.03
<b>Roots:</b>						
No. per acre	19,560	19,390	16,620	15,470	17,760	338.8
Percentage	110.1	109.2	93.6	87.1	100.0	1.9



Treatments C. and D. have resulted in a significant depression in yield both for roots and tops; further, treatment D. is significantly below C. in root yield.

We thus have a repetition of the results obtained in 1926, although the depression in yield with comparable treatments (C. in Table III, S. in Table I) is not so large. There is the further result that the second shallow rotary cultivation in Series D. was associated with additional drop in the yield of roots. The immediate explanation of these results would be to ascribe the reduction in yield to rotary cultivation, but a fuller examination puts the matter in a different light. In the first place, treatment B.

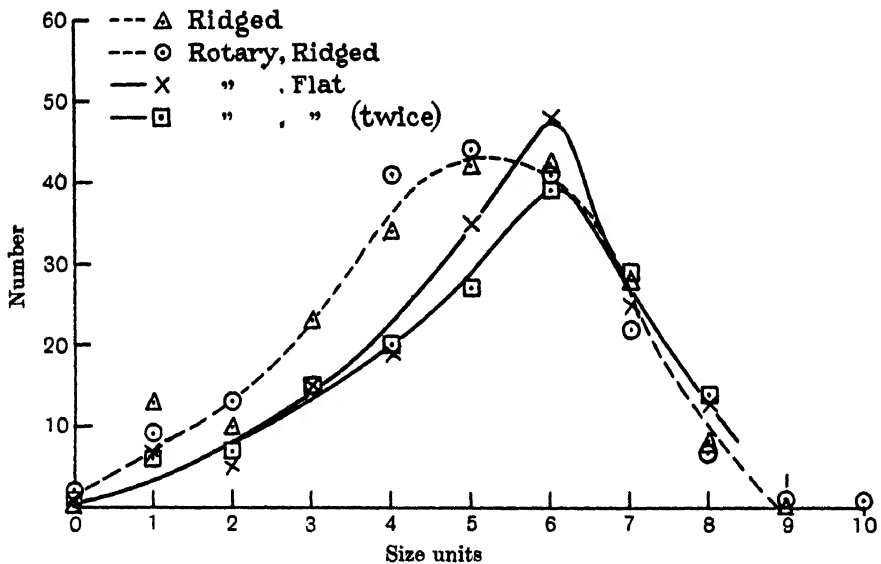


Fig. 4. Rotary cultivation 1928 experiments. Size distribution of roots.

has given yields equal to the standard treatment A., although it included rotary cultivation. In the second place the reduction in 1926 was definitely associated with a capping or hardening of the soil, and there were no signs of this in 1928. In the third place, an examination of Table III shows that the yield in each of the four treatments is closely proportional to the actual number of roots on the plot. The closeness of this agreement is well shown by comparing the second and last lines in the table. The differences in yields may therefore be ascribed to differences in number of roots, more especially as Fig. 4 shows that the size distribution of the roots was much more uniform over the different cultivation treatments than was the case in 1926 (Fig. 2). A number of possible causes suggest themselves for this decrease in the number of roots but,

in the absence of the necessary data, no definite explanation can be advanced.

*The 1929 experiments.* The similarity between the 1926 and 1928 results made it highly desirable that the residual effect of 1927 should again be looked for, and it was therefore decided to follow the general plan of the 1927 experiments. The weather prevented winter ploughing, and the whole area was ploughed, using horse teams, early in March. The land was then tooth harrowed immediately before and after drilling with barley on March 14th. Manures were sown on March 27th and consisted of 2 cwt. super-phosphate, 1 cwt. muriate of potash and 1 cwt. sulphate of ammonia, per acre. This set of plots will be referred to below as the Old Set.

At the same time a second set of 16 plots (New Set) was established only a few yards from the Old Set, on land that had also carried swedes in 1928. This area had also received 16 tons per acre of dung as well as artificials, and was therefore in a better state of fertility than the Old Set plots. The purpose of the New Set was to test rotary cultivation as a seed-bed preparation for barley. The land had had no winter ploughing after the swedes were removed. The comparison was, therefore, the direct one between spring ploughing and harrowing, and rotary cultivation, and there were eight plots for each of the two cultivation treatments. The operations were carried out, and the land was manured and sown at the same time as the Old Series. The season was an excellent one, and good growth and yields were obtained both on the Old and New Sets.

The results for the Old Set are summarised in Table IV.

Table IV. *Old Set. (Barley, 1929—all plots ploughed and harrowed.)*

Series	A.	B.	C.	D.		
Cultivation treatment in 1928	Ridged	Rotary cultivation and ridged	Rotary cultivation left flat	As C. with second rotary cultivation	Mean	Standard errors
Grain:						
Cwt. per acre	27.2	27.3	24.8	28.4	26.9	1.11
Percentage	101.1	101.2	92.3	105.5	100.0	4.13
Straw:						
Cwt. per acre	35.0	37.3	33.5	41.2	36.7	1.72
Percentage	95.2	101.4	91.3	112.1	110.0	4.67

There is no significant difference between the old rotary cultivation and ordinary cultivation plots, as far as the yield of grain alone is concerned, and the striking observation of 1927 (Table II) is therefore

not repeated. For the straw the differences just approach the level of significance. It is interesting to note that, while series C. is still below the mean, as in 1928, series D. gives the highest yield and appreciably exceeds A. and B. In 1928, treatment D. was significantly below C., which in turn was below A. and B. We thus have a partial repetition of the 1927 results, in that an area which gave under a rotary cultivation treatment the lowest yield of swedes, gives under ordinary cultivation in the succeeding year the highest yield of barley straw and grain.

In the New Set, counts were made of the number of plants present about one month after sowing. The usual significant difference in favour of the rotary cultivation was observed. It was also noticed that higher numbers of plants occurred in the centre of the plots than on the sides, in both the ploughed and rotary cultivated series. In the ploughed series this difference is understandable. In distinction to the 1928 experiments, where a one-way plough was used, the ploughed plots were ploughed in narrow lands in 1929, in the ordinary manner. In consequence the ridge with its deeper tilth ran down the middle of each ploughed plot and the furrows along each edge. This explanation will not serve for the rotary cultivated plots, as the work was begun at one side of the plot and proceeded regularly to the other side, and the depth of tilth was therefore sensibly uniform.

During the later stages of growth no differences could be seen, and the small differences in early growth recorded above were doubtless soon levelled by tillering of the plants. An excellent crop was obtained, and the yields are shown in Table V.

Table V. *New Set. (Barley, 1929—no autumn ploughing.)*

Series Cultivation treatment in spring	E. Ploughed and harrowed	F. Rotary cultivation left flat	Mean	Standard errors
Grain:				
Cwt. per acre	30.50	29.93	30.22	0.575
Percentage	101.0	99.0	100.0	1.90
Straw:				
Cwt. per acre	44.93	43.75	44.34	1.192
Percentage	101.3	98.7	100.0	2.69

Table V shows that both rotary and ordinary cultivations gave excellent crops, there being no significant difference between them. The yields are appreciably higher than those shown in Table IV. The difference is attributable to the residual value of the dung given to this area in 1928.

*Summary of the crop results, 1926-9.*

Rotary cultivation as a means of seed-bed preparation for swedes and barley gives earlier and better germination of seed, and superior early growth. This initial superiority, however, is not maintained. In the case of swedes actually a lower yield is given, which was definitely associated with a hardening of the soil during the growth period in the 1926 experiments, but not in the 1928 results. Rotary cultivation is satisfactory for barley, and yields are obtained equal to those given with ordinary cultivation.

The reduction of yield of swedes due to rotary cultivation is followed by a marked increase in the yield of the succeeding crop (barley), when the plots in question are cultivated in the ordinary way.

## SECTION II. MEASUREMENTS OF SOIL COMMINUTION.

This section describes the measurements made to ascertain whether there were any marked differences produced on the soil by the various cultivation implements used in the four years' experiments. The method selected was to measure the proportion of soil remaining on each one of a set of different sized sieves, samples of soil being taken for this purpose immediately before and after the passage of the implement. In any experiment a considerable number of samples is needed from each plot on account of soil heterogeneity.

Four sieves were used: three had square apertures of sides of 1.5 in., 0.5 in., and 0.25 in., while the fourth was the usual 3 mm. round hole type, and may with sufficient accuracy be taken as equivalent to a square meshed sieve of 0.1 in. side. The successive passage of a soil sample through this set of sieves gave five groups, (a) to (e), group (a) consisting of lumps not passing the 1.5 in. sieve, and group (e) of soil crumbs small enough to pass the 0.1 in. sieve. Samples were taken with a spade, a cube of soil being isolated by vertical cuts with as little disturbance as possible. The spade was then carefully inserted under the cube, which was transferred with the minimum disturbance to a sheet of stout paper and taken to the sieves. The sample was passed progressively through the set of sieves, a gentle oscillation of each sieve being maintained until no more soil passed through. Each group was then weighed and expressed as a percentage of the total weight of the sample. The weights are not corrected for the moisture in the soil. Measurements of the moisture content were always taken, and showed but little variation during any test. The comparisons of the sieving results before and after

cultivation are not, of course, affected by including the moisture content. Further, the average moisture values in the successive years are not sufficiently different from each other to invalidate direct comparisons of the sieving results from one year's experiments to another. The stony nature of the land presented a difficulty, especially in groups (a) and (b), which sometimes contained an appreciable proportion of stones. It was decided to remove by hand from these groups all stones larger than about  $\frac{1}{2}$  in. in diameter before weighing the sample. In the tables below, besides the percentages of the five fractions, there is given a "surface area" figure. This is a rough measure of the total surface area of the individual pieces making up the whole sample, and serves as a "single value" measurement for specifying the amount of comminution in any sample. To obtain this figure it was assumed that the pieces were cubes, and that the average size of these lumps of soil on any sieve was midway between this sieve aperture and the one immediately above. The sides of the cubes were therefore approximately 0.05 in., 0.2 in., 0.4 in., 1 in., while for the fraction on the largest sieve (whose contribution to the total area is small in any case) a value of 2 in. was arbitrarily assigned. The surface area of any group is then proportional to the number of pieces multiplied by  $A^2$ , where  $A$  is the length of the cube edge; the number of pieces is in turn proportional to the percentage of that group divided by  $A^3$ . Hence, the surface area is proportional to the percentage of that group divided by  $A$ .

*The 1926 measurements.* As already mentioned in Section I, the soil was in excellent condition, and was ready to fall down into a good tilth under almost any form of cultivation. In fact, sampling before the cultivations had to be done with considerable care, otherwise the soil block fell to pieces in the process. The percentages of the various groups isolated by the sieves are given, together with the surface figure, in Table VI.

Table VI. *Seed-bed for swedes, 1926.*

Group	Before cultivation (ninefold replicates), percentage of each group			After cultivation (ninefold replicates), percentage of each group		
	Rotary S.	Flat F.	Ridging N.	Rotary S.	Flat F.	Ridging N.
(a)	10.9	7.5	14.1	1.9	4.3	6.0
(b)	27.9	32.9	27.0	21.8	24.6	20.8
(c)	17.7	19.6	18.8	17.7	18.8	15.6
(d)	24.4	13.2	23.5	27.6	26.7	26.2
(e)	19.1	16.8	16.6	31.1	25.6	31.5
"Surface"	581	538	531	827	719	824
Percentage increase in surface after cultivation :—				42	34	55

Inspection of the before-cultivation results shows a reasonably close agreement in the proportions of the different groups present, indicating that the ninefold replicates taken (three on each of the triplicate plots) are adequate. The surface figures do not agree so closely, since they are sensitive to small changes in the amount of fractions (*d*) and (*e*). Comparison of the group percentages before and after cultivation shows that, within the limits of the sieving method, there is little to choose between the three methods of cultivation in efficiency of disintegration of the soil. However, the surface figure and the corresponding percentage increases offer some suggestion that ridging was most effective, followed by rotary cultivation and flat seed-bed treatment, in the order named. This sequence, it will be found, is repeated in the later experiments.

Table VI shows that rotary cultivation did not produce a much finer tilth than traditional methods, even in this soil which was peculiarly ready to disintegrate. It would, therefore, appear that the frequently made assertion that rotary cultivation produces too fine a tilth is incorrect. It certainly produces a much looser tilth, for reasons already given, and probably this appearance of the soil after cultivation has led the practical man to interpret it wrongly as a finer tilth.

Frequent measurements of moisture content were made during the season, and a recording thermometer was installed at the 6 in. depth on each of the three plots in the central block, further temperature records being taken on selected occasions at different depths with ordinary mercury thermometers. Examination of the results fails to show any close connection between them and the observed behaviour of the crop, with the exception of the temperature records for the first weeks after sowing. The rotary cultivation plot gave higher daily minima and maxima than either of the other plots, and the earlier germination is doubtless due to this.

The remarkable slowing up in growth already discussed must be attributed, therefore, to the consolidation of the soil, and not to any extra fineness in the tilth, or to moisture and temperature factors.

No further measurements were made on the plots in 1927.

*The 1928 measurements.* These results form a useful contrast to those of 1926. They refer to a different field which, however, is practically identical in mechanical composition to the original one. At the time of the cultivations, the land was still suffering from the wet season of 1927 and the winter of 1927-8, and its physical condition was distinctly inferior to Sawyer's field in 1926. The difference was easily apparent

during the actual cultivations and is well brought out by the sieving tests, recorded in Table VII.

Table VII. *Seed-bed for swedes, 1928.*

Before cultivation (twelffold replicates): percentage of each group.				
Series	A.	B.	C.	D.
Cultivation treatment	Ridged	Rotary cultivation and ridged	Rotary cultivation left flat	As C. with second rotary cultivation
Group				
(a)	60.2	61.8	58.2	60.8
(b)	11.3	11.2	13.9	12.5
(c)	11.1	10.4	11.2	11.4
(d)	8.0	7.4	7.8	6.7
(e)	9.3	9.3	8.8	8.5
"Surface"	295	291	286	274
After cultivation (twelffold replicates): percentage of each group				
Series	A.	B.	C.	D.
Cultivation treatment	Ridged	Rotary cultivation and ridged	Rotary cultivation left flat	As C. with second rotary cultivation
Group				
(a)	28.9	14.8	48.5	42.3
(b)	17.7	20.1	12.3	13.8
(c)	20.2	23.6	14.4	14.7
(d)	13.3	18.3	11.0	11.8
(e)	19.8	23.2	13.8	17.3
"Surface"	546	640	403	477
Percentage increase in surface after cultivation :—	85	120	41	74
				58

Comparison of the before-cultivation figures with those of Table VI for 1926 shows the physical difference that existed between the two soils. The outstanding feature is the large percentage of group (a) in 1928, and the low values for the two finest groups (d) and (e). A comparison of the after-cultivation results 1928, with the before-cultivation figures 1926, is even more striking. Except for group (a) in series C. and D. the general run of the figures in the two years, including the surface values, is much the same. It appears, therefore, that in 1926 the gentle disturbance incurred in taking the sample and sieving it gave the same final result as the drastic operations of rotary tillage, or of ridging, in 1928. The two tables clearly show the great influence of climatic conditions on the tilth of the soil.

Examination of the actual effects of the cultivation operations in 1928 also show important differences. Further evidence that rotary

cultivation does not necessarily produce a finer tilth than ordinary methods is given by the high figures for group (a) in series C. and D. in comparison with series A.

A further point of importance is the demonstration of the great value of a ridging or bouting implement in breaking up unkindly soil. It is at least as effective as rotary cultivation, while the combination of the two is naturally the most efficient of the three treatments. The net effect of the combination as judged by the percentage increase in the surface area figures is an additive one, although this conclusion is made less certain by the wide difference between series C. and D. (41 per cent. and 74 per cent. respectively). The difference is a little disturbing as treatments C. and D. were identical, the second rotary cultivation on D. being done at a later date. Further, the results are the average of twelvefold replicates in each case.

As already mentioned, there was no capping or hardening of the soil in 1928; the coarser tilth in this year, which is well brought out by the sieving results, is the probable reason for its absence.

*The 1929 measurements.* There were two separate experimental areas in this year. One set consisted of the 1928 area, which was ploughed and harrowed throughout, and sown to barley to see whether the reduced yield of swedes on the rotary cultivation plots would again be followed by an increased yield of barley. The other set was a direct comparison on adjacent land of rotary and ordinary cultivation as a seed-bed preparation for barley. The sieving results are shown in Tables VIII and IX respectively.

Table VIII. *Barley (Old Set)*, 1929.

(Cultivation treatments in 1928 are shown in Table VII.)

Series Group	Before ploughing.			
	A.	B.	C.	D.
(a)	25.1	36.1	35.4	29.3
(b)	22.1	19.0	18.3	22.3
(c)	10.1	9.0	9.1	9.8
(d)	28.3	23.4	24.9	24.6
(e)	14.4	12.5	12.3	14.0
"Surface"	490	426	429	464
After ploughing.				
Group				
(a)	20.8	24.3	24.4	30.1
(b)	23.0	21.6	20.0	19.7
(c)	12.8	12.2	13.8	12.8
(d)	35.0	31.1	31.0	28.6
(e)	8.4	10.8	10.8	8.8
"Surface"	409	436	438	386



Table IX. *Barley (New Set), 1929.*

Series	E.	F.	E.	F.
Cultivation treatment	Ploughed and harrowed	Rotary cultivation	Ploughed and harrowed	Rotary cultivation
Group	Before cultivations		After cultivations	
(a)	37.6	36.7	29.5	13.2
(b)	21.7	22.4	14.6	17.1
(c)	9.5	9.6	10.5	13.2
(d)	24.7	22.9	32.5	43.6
(e)	6.5	8.4	12.9	12.9
"Surface"	318	346	476	533
Percentage increase in surface after cultivation :—			50	54

The before-ploughing figures in Table VIII compared with the after-cultivation values for the same area in Table VII show that only a moderate amount of consolidation had taken place in the interval. The severe cold spell in February 1929 probably counteracted to some extent the general consolidation of the soil during the winter. The figures also show that any difference due to the different methods of cultivation in 1928 had been evened up by the time of the 1929 ploughing.

Table VIII also brings out the surprising result that ploughing did not, in the particular conditions of this experiment, produce any appreciable shattering effect on the soil, whether one takes the percentage distribution of the sieve fractions or the surface figures as the criterion. There is a suggestion that the plough produced a sub-division of the largest sized lumps, and at the same time an aggregation of the smallest lumps into larger ones, for group (d), particularly, is increased by ploughing and groups (a) and (e) are decreased. A decrease in group (a) would certainly be expected, no matter what physical state the soil was in, while the decrease of group (e) could be explained by the compressing effect of the tail-end of the mould board on the furrow slice as it is pressed into position. But when the before- and after-ploughing figures for the New Set of plots (Table IX) are compared with those just discussed, it is observed that, in this case, ploughing did have an effect, amounting to about 50 per cent. increase in the surface figure. This area had received a liberal supply of organic manure in 1928, and was hence in better tilth than the Old Set.

On the New Set of plots, the effect of rotary cultivation is seen to be little different from ploughing, except that the proportion of group (a) is much less. This result, which was not obtained in 1928, is probably also due to the better inherent tilth in 1929, following the application of organic manure in the previous year.

*Summary of Section II.*

The outstanding feature of the results is the predominant effect of meteorological factors on the physical condition of the soil. If the soil is in good condition, almost any form of cultivation will produce a satisfactory tilth.

The general effect of rotary cultivation is to leave a smaller proportion of large lumps and hence a more uniform tilth, but it does not produce a much finer tilth than the traditional forms of cultivation. It leaves a much looser or "puffed up" seed-bed, the appearance of which has no doubt given rise to the frequent but incorrect assertion that rotary cultivation produces too fine a tilth on loams or heavy soils.

The ridging or bouting plough is shown to be an effective implement for breaking up soil in poor physical condition, and is appreciably superior to the ordinary plough. A ridging body attached to a rotary cultivation implement is even more effective.

The ordinary plough does not produce much comminution of the soil unless it contains a good supply of humus, and is therefore in good physical condition. At other times the tendency is to increase the percentage of medium-sized lumps, at the expense of the largest and smallest, the latter becoming aggregated by pressure from the tail-end of the mould board as the furrow slice is pressed into position.

## GENERAL DISCUSSION.

This section briefly supplements the detailed discussion given in Sections I and II.

The outstanding feature of the four years' experiments on rotary cultivation for preparing a seed-bed for spring crops is the contrast between the beginning and end of the growth period. Practically without exception, quicker and better germination, and more rapid early growth, have followed from this method of cultivation. But, again without exception, this initial advantage has either been lost or even replaced by a reduced yield at harvest. The absence of any significant difference in the yield of barley between the rotary cultivated and normally cultivated plots is not surprising; Eden and Maskell<sup>(1)</sup> have shown for wheat that a reduced germination percentage implies a greater available root range for the survivors, and is thus associated with more abundant tillering, which in consequence levels up the final yield.

In the case of swedes, however, where this effect does not enter, rotary cultivation was often associated with a reduced yield, of consider-

able magnitude in the 1926 experiments, and smaller but still significant in the 1928 experiments. This crop is known to be sensitive to soil conditions, and possibly the 1928 results may be due to some small factor that can be removed after further experience of rotary cultivation. The cause of the large reduction in yield in 1926 has already been discussed. It was due to an excessive and extensive hardening or "capping" of the soil on the rotary cultivation plots, which, no doubt, would have seriously affected most agricultural crops.

The results of the Rothamsted experiments are confirmed by the work of Gade<sup>(4)</sup> in Germany. His experiments were made on various soils and crops, including winter wheat, barley, oats, sugar beet and potatoes. The yields for sugar beet were the same on the rotary cultivated and ploughed plots; with the other crops a slight and insignificant increase was given; in one of the potato experiments a significant increase was obtained on the rotary cultivated plots. Gade also confirmed the initial superiority of rotary cultivated soil, and was able to show by measurements of pore volume that it existed in a much looser condition than soils cultivated by normal methods. Owing to the presence of numerous stones and flints in the Rothamsted soil, no attempt was made to take volume measurements. The effect of rotary cultivation was, however, visible to the naked eye. The soil rapidly settles into a compacter tilth, which in some cases (*e.g.* the 1926 experiments) may be deleterious to the growing crop. In Gade's experiments the initial lightness of structure had disappeared after 100 days. The absence of any marked benefits in yield after rotary cultivation is, therefore, not surprising. On the other hand, there is no foundation for the belief that a very fine tilth is normally produced by the rotary cultivator. Such a tilth would almost always be disadvantageous on heavy loams and clay soils. The action of the tines ensures that few large lumps of soil are left and the tilth is, therefore, more uniform, but there is no great preponderance of the smallest sized lumps.

The experiments clearly show that the tilth of medium and heavy soils depends predominantly on the weather conditions, the type of implement employed having normally only a secondary influence. The rotary cultivator is of greatest service when the soil is not in good condition, as is the case after a wet and open winter. In these circumstances, it is difficult to refine the soil to a good tilth, and here rotary cultivation shows to the best advantage, particularly if it is combined with the operation of ridging, which is in itself an effective method in the same conditions.

## SUMMARY.

Experiments extending over the four years, 1926-9 inclusive, have been carried out on a heavy and stony loam soil to compare rotary cultivation with normal methods for the production of a seed-bed. The work has been confined to spring-sown crops—swedes and barley—and to spring cultivations.

Rotary cultivation gives earlier and better germination of seed, followed by better early growth.

In every experiment, however, the final yield has either been no better, or else significantly below that obtained from the plots cultivated in the usual way. The barley crop gives equally good yields under the various soil cultivation treatments; although there is better germination under rotary cultivation, the plants on the remaining plots, having greater root range, throw out more tillers and thus level up the yield. The swede crop did not do so well under rotary cultivation, in spite of better early growth. In the 1926 experiments this was due to an extensive hardening or "capping" of the soil on the rotary cultivation plots but, although this effect was absent in the 1928 experiments, a reduced yield was still obtained.

In addition to records of yield, measurements were made of the amount of soil pulverisation or comminution produced by the various implements. Samples of the soil before and after cultivation were passed through a set of sieves, of different mesh, and the proportion of soil on each sieve was recorded.

The meteorological factors exercise a predominating influence on the physical condition of the soil; the influence of the implement is secondary.

Rotary cultivation is most effective on unkindly soil, *e.g.* after a wet and open winter, as it leaves a smaller proportion of large lumps.

It does not produce an appreciably finer tilth than the usual implements, but it leaves the soil in a much looser condition, and thus encourages the early germination of seeds.

The looser tilth becomes more compact with time, and the initial advantage of rotary cultivation then disappears.

If a ridging body is attached behind the rotary cultivator, a further comminution of the soil is produced. The operation of ridging is itself surprisingly effective in breaking up land in poor physical condition.

Ploughing does not necessarily produce any comminuting action on the soil unless it is well supplied with humus and is, therefore, in good

inherent tilth. When the soil is in poor condition, there is a tendency for the smallest soil crumbs to be aggregated into larger ones, probably owing to the compression of the soil by the tail-end of the mould board. This effect counteracts the increase of surface due to the sub-division of the largest sized lumps.

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## THE ELECTRICAL CONDUCTIVITY OF AQUEOUS SOIL SUSPENSIONS AS A MEASURE OF SOIL FERTILITY.

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(With Five Text-figures.)

### INTRODUCTION.

IN a previous paper one of the authors (6) confirmed the observations of Atkins (1) that there was a rapid increase in the electrical conductivity of aqueous extracts of soil as the extraction was prolonged in the case of fertile soils, while there was little or no increase in the case of infertile soils. It was further shown that, for soils collected at the same time from adjacent plots, the increases in conductivity after 8 to 10 days corresponded with the crop yields from these plots. From this it seemed probable that if samples of soil were taken at intervals and at known dates from plots under continuous cultivation, the decrease in fertility would be shown by corresponding decreases in the electrical conductivity. In fact, the other author (8), who had found that the specific conductivity of a soil extract is a measure of the fertility of the soil, obtained indications of decreases in conductivity in Nigerian soils under cultivation; the results were not conclusive, probably because the period over which the soil samples were collected was too short.

The soil samples that have been taken at intervals from the Rothamsted classical plots and carefully preserved in bottles are probably the most valuable material for testing the relationship between electrical conductivity and soil fertility. It was, therefore, decided to measure the "initial conductivity," i.e. specific conductivity of a 1 to 5 aqueous suspension determined at 25° C. in mhos (i.e. reciprocal ohms) and the "7 days' increase," i.e. the rise in specific conductivity of the same aqueous suspension after standing for 7 days in the thermostat at 25° C.

## 2 *Electrical Conductivity of Aqueous Soil Suspensions*

### SOIL SAMPLES USED.

Stored soil samples from the following Rothamsted plots were examined:

- |                        |   |
|------------------------|---|
| (1) Broadbalk Plot 2B  | Manured with 14 tons of dung per acre.  |
| (2) Broadbalk Plot 3   | Unmanured.  |
| (3) Broadbalk Plot 7   | Manured with 412 lb. of sulphate of ammonia, 3½ cwt. of super-phosphate, 200 lb. of sulphate of potash, 100 lb. of sulphate of soda and 100 lb. of sulphate of magnesia per acre. |
| (4) Hoosfield Plot 1-0 | Unmanured since 1852 and under barley every year.   |

All the Broadbalk plots, except Plot 3, receive the same manure annually, and have been under wheat since 1843. Previous to that year the plots were all treated alike. The crops grown and the manures applied for the previous 5 years were turnips with farmyard manure in 1839, and subsequently barley, peas, wheat and oats without manure.

### EFFECT OF STORAGE ON CONDUCTIVITY.

Since most of the soil samples were stored for many years, differences in the measurements, if any, might be due to (i) seasonal differences, since all the samples were not collected at the same time of the year, or (ii) differences in the period of storage. The first of these will be fully dealt with by the first author(7): it will be shown that there is no significant seasonal variation in the measurements for the Rothamsted soil from the unmanured plot, but the measurements for the manured plots are affected temporarily by the application of manure and the growth of the crop. Consequently the main conclusion is drawn from the measurements on unmanured soil.

With regard to the second factor there was, of course, no information whether prolonged storage might result in some slow changes likely to affect the measurements. However, as the soil samples had always been stored in the air-dry condition, in air-tight bottles, it appeared reasonable to assume that any such changes would occur mainly during the initial stages of storage. The following experiments were, therefore, conducted to study the influence of the initial storage on the measurements. The total duration was slightly over nine months. Composite samples of soil, each of which was a mixture of three separate holes (0-9 in.), were taken from three Broadbalk plots, 2B, 3 and 7. They were dried in the air at room temperature for 8 to 10 days, passed through a 1 mm. sieve and stored in bottles. The conductivities of the aqueous suspensions of the air-dry samples thus stored were determined in duplicate as follows: 100 c.c. of conductivity water were added to 21.2 gm. of air-dry soil (i.e.



20 gm. of oven-dry soil) in a bottle and the mixture was shaken for 1 hour in an end-over-end shaking machine and, after subsequent standing for 40–45 minutes in the thermostat at 25° C., its conductivity was measured. The increase in conductivity after 7 days' standing was

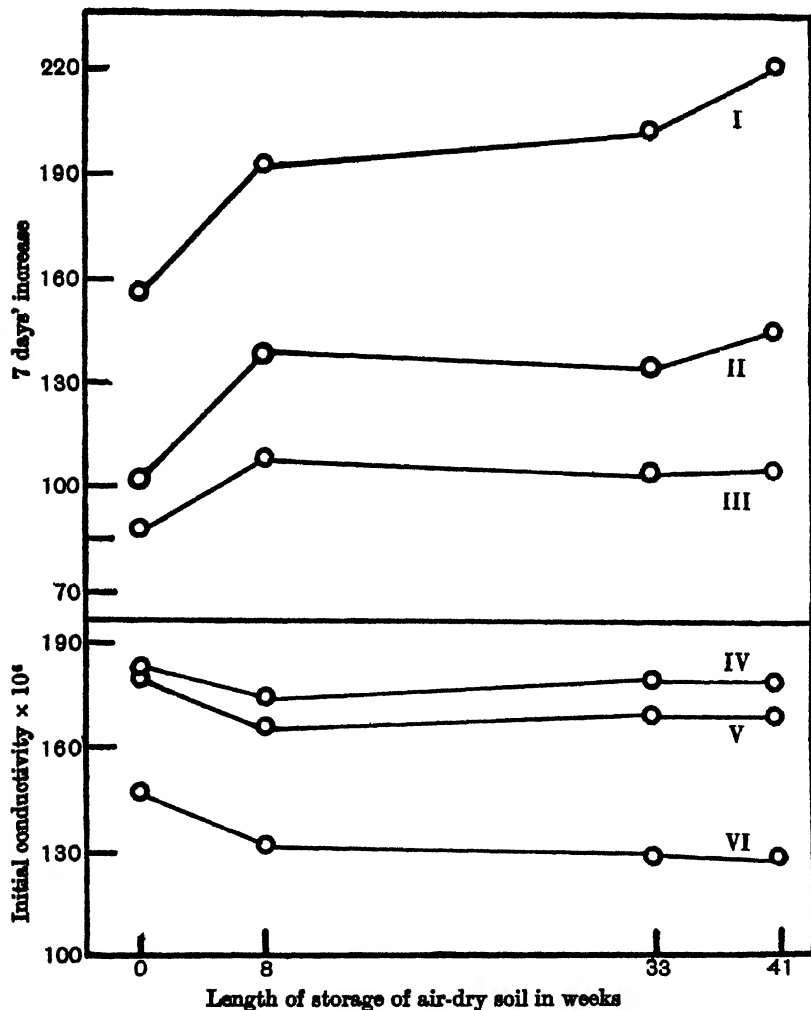


Fig. 1. Broadbalk field.

Curves I and IV, Plot 2. Farmyard manure.

„ II and V, Plot 7. Minerals.

„ III and VI, Plot 3. No manure.

also measured. The technique will be fully described by the first author (7). The results are given in Fig. 1.

The initial conductivity measurements for the three Broadbalk samples (Fig. 1) decreased slightly during the first few weeks' storage,

#### 4 *Electrical Conductivity of Aqueous Soil Suspensions*

but remained remarkably constant from the eighth week till the end of the experiment. It is evident therefore that prolonged storage has very little effect on the initial conductivity of air-dry soil. In the case of unmanured soil or that treated with inorganic fertilisers the 7 days' increase became larger with continued storage, but attained a fairly constant maximum value in the course of the first 2 to 4 months. On the other hand, in the case of the dunged soil, the maximum was not reached even after storing for 41 weeks. But as the value was increased by 37 units after the first 8 weeks' storage, while on continued storage for a further period of 33 weeks it was increased by 28 units only, it may be expected that, on continuing the experiment further, a maximum value would have been reached, probably within a few months.

Parallel experiments were also made on samples taken from another field; they gave results closely similar to the Broadbalk samples.

In view of the above observation it is assumed that, since the old soil samples forming the subject of the present investigation have been stored for many years, they have already attained the maximum values and are therefore comparable.

#### METHODS.

In 1927 fresh soil samples were collected from the Broadbalk plots, 2B, 3 and 7, and Hoosfield plot, 1-O, dried in air for 10-12 days, passed through 1 mm. sieve and then preserved in corked bottles for over 1 year so as to make them comparable with the other old stored samples from these plots. Since most of the other samples were available in very small quantities, the following modification in the preparation of the soil suspensions was adopted: 10 gm. of air-dry soil were taken in an 8 in. Pyrex test-tube to which 50 c.c. of conductivity water were added. The mouth of the tube was closed by means of a paraffined cork, and the mixture was thoroughly shaken for half a minute. It was then allowed to stand in the thermostat at 25° C. and, after 24 hours, the initial conductivity was determined, using a dip-electrode. The latter was withdrawn and the tube containing the suspension was left in the thermostat for 8 days. On the fifth day the suspension was shaken once for half a minute, and on the eighth day the final conductivity was measured, using the same dip-electrode. The difference between the final and initial conductivity gave the "7 days' increase."

#### *Broadbalk Plot 3.*

In Fig. 2 the measurements of initial conductivity and 7 days' increase are given for the stored samples from this plot, together with the

crop yields. The latter are given in two different forms. One (Curve III) is the actual yield of the particular year for which the stored sample was available, while the other (Curve IV) is the average yield for three consecutive years, viz. the particular year of sampling and the years immediately preceding and following it.

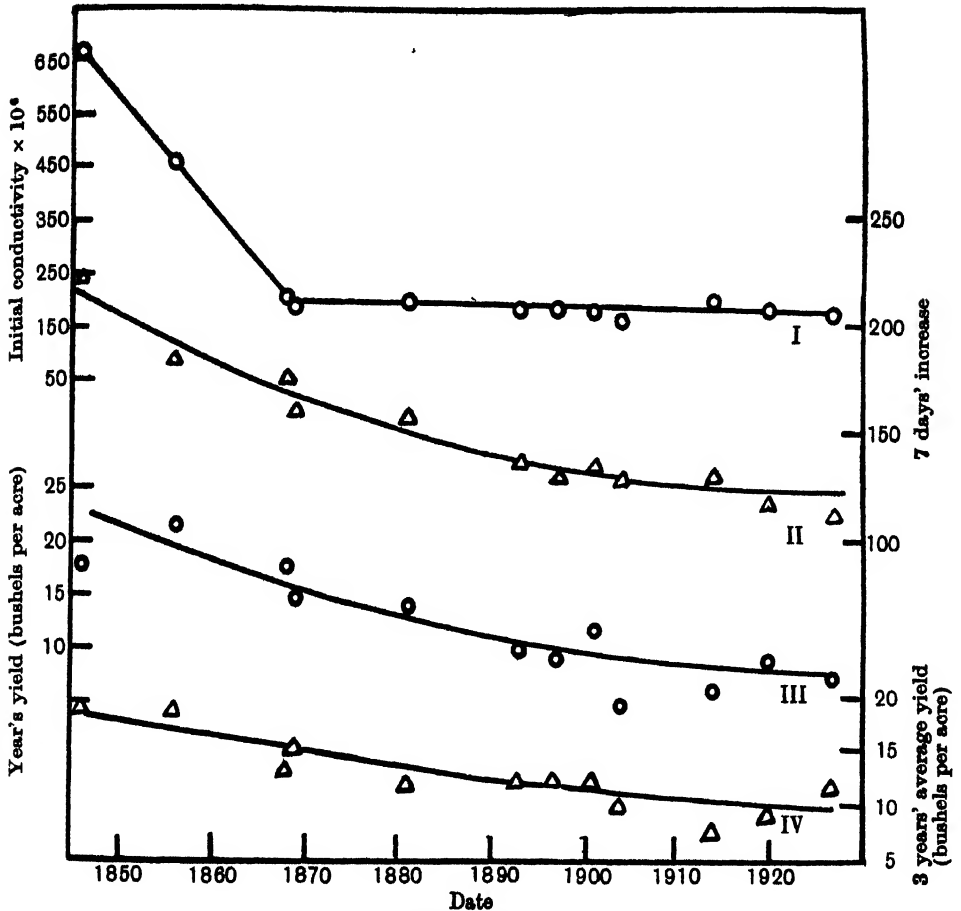


Fig. 2. Broadbalk Plot 3. No manure.

- I. Initial conductivity of air-dry soil samples collected during the years shown.
- II. Seven days' increase of air-dry soil samples collected during the years shown.
- III. Yield of wheat in bushels per acre for the years shown.
- IV. Average yield of wheat in bushels per acre for three years, viz. the year shown and the year preceding and following it.

Under continuous cropping the initial conductivity (Curve I) of Plot 3 steadily decreased from the year 1846 to 1869. After this date sensibly constant values are given. It has been observed by the senior author(7) that the initial conductivity of a plot, which increases on the

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application of manures, comes down to the value previous to manuring in the course of a few months. Consequently, as the above plot was not manured after 1839, the high initial conductivities of the samples of 1846 and 1856 were probably not due to the residual effect of previous manuring. The soluble salt content of the plot, which was therefore normally high up to 1856, was gradually brought down under continuous cropping to a certain low value in 1869, and afterwards changed very little over a period of 58 years. This shows that the crop can deplete the soil of its soluble salts only up to a certain point. It is interesting to note that Burd and Martin(2), while studying changes in the soil solution, observed that in soils (contained in vessels) which have been cropped for some years, the initial concentration of the solution in any given growing season returns to its original magnitude by the beginning of the following season.

Fig. 2 shows also that there is only a very general relation between the initial conductivity and crop yield, viz. the higher initial conductivities before 1868 are associated with higher yields. The distinct tendency for a gradual decrease in the yield of wheat under continuous cropping after 1868 (as seen in the 1-year yield figures) is not reflected in the initial conductivity. On the other hand, a close relationship is evident between the 7 days' increase figure and that for the 1 year's crop (Curves II and III), both decreasing with time under continuous cropping. The correlation coefficient between the two sets of values is  $+0.859$ .

### *Hoosfield Plot 1-O.*

The results are given in Fig. 3. The first sample from this plot was not taken until after 30 years of continuous cropping without manure, hence much of the information regarding the relationship between the measurements of conductivity and crop yield is probably lost, since it has been shown above that the changes both in the measurement and the yield of Broadbalk Plot 3 are very small after a period of 30 years' cropping. With the exception of the two high measurements for the samples of 1889 and 1913, the initial conductivity (Curve I) for this plot shows a regular slow decrease. It is difficult to explain the reason for the two high measurements mentioned above. The plot was fallowed in 1912, but whether this was in any way connected with the increased measurement in 1913 is not known. But it may be mentioned that continued fallowing of Rothamsted plots was not found(7) to affect the measurements.

The yield of this plot was found to fluctuate very greatly, but the 3 years' average figures show a gradual falling off in the yield. On the whole there is a general parallelism between this set of figures and that

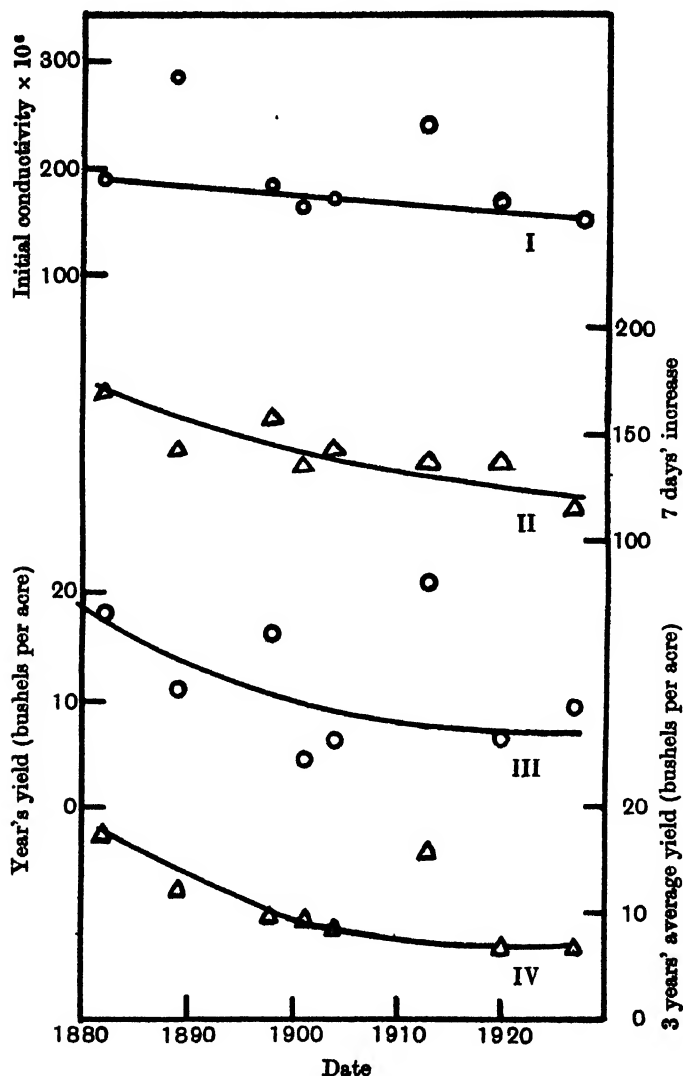


Fig. 3. Hoosfield Plot 1-O. No manure. For explanation of numbering see Fig. 2.

for the 7 days' increase. The high yield obtained in 1913 can be attributed to the previous fallowing as shown below.

Year	1910	1911	1912	1913	1914	1915
Yield of barley in bushels per acre	9.9	4.9	Fallowed	21.1	10.4	9.5

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### *Broadbalk Plots 7 and 2B.*

The results for these two plots are given in Figs. 4 and 5. For Plot 2B the earliest sample available was that of 1868, and for Plot 7 that of 1881. Here again Curve II in each of the two figures shows a

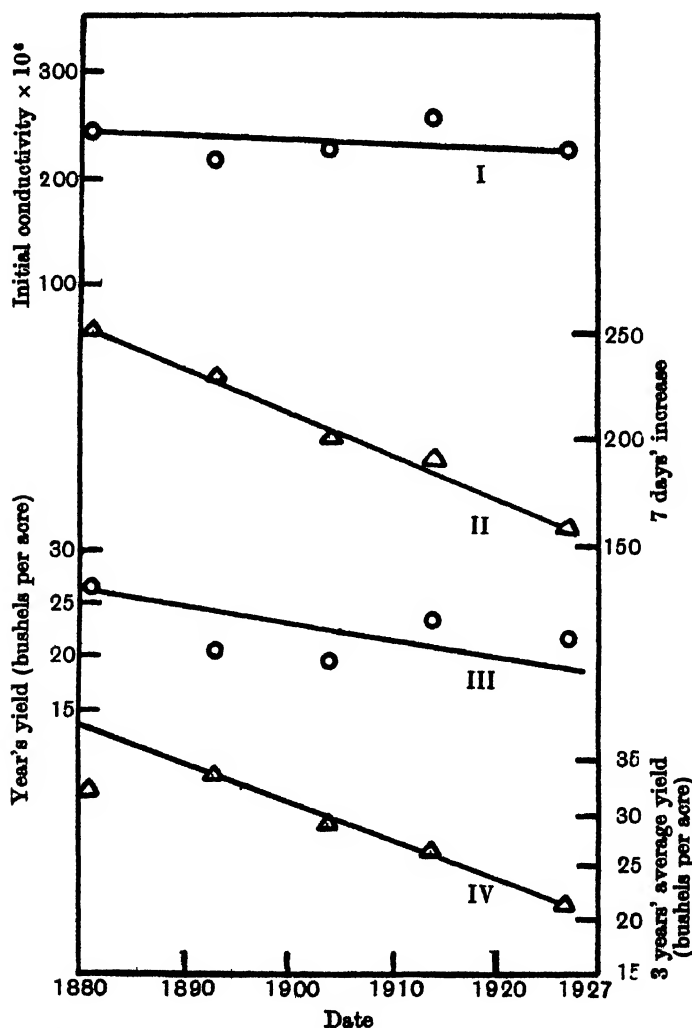


Fig. 4. Broadbalk Plot 7. Inorganic manures. For explanation of numbering see Fig. 2.

distinct tendency in the 7 days' increase to fall off, but the number of stored samples available for examination is too small to warrant any detailed discussion. One fact, however, is fairly obvious; by yearly application of manure the 7 days' increase of both plots was maintained throughout at a higher level than that of the unmanured plot (Curve II,

Fig. 2). Also this difference in the levels was greater when dung was applied (Plot 2B) than when inorganic fertilisers only were used (Plot 7).

The results show that the initial conductivities of the earliest samples

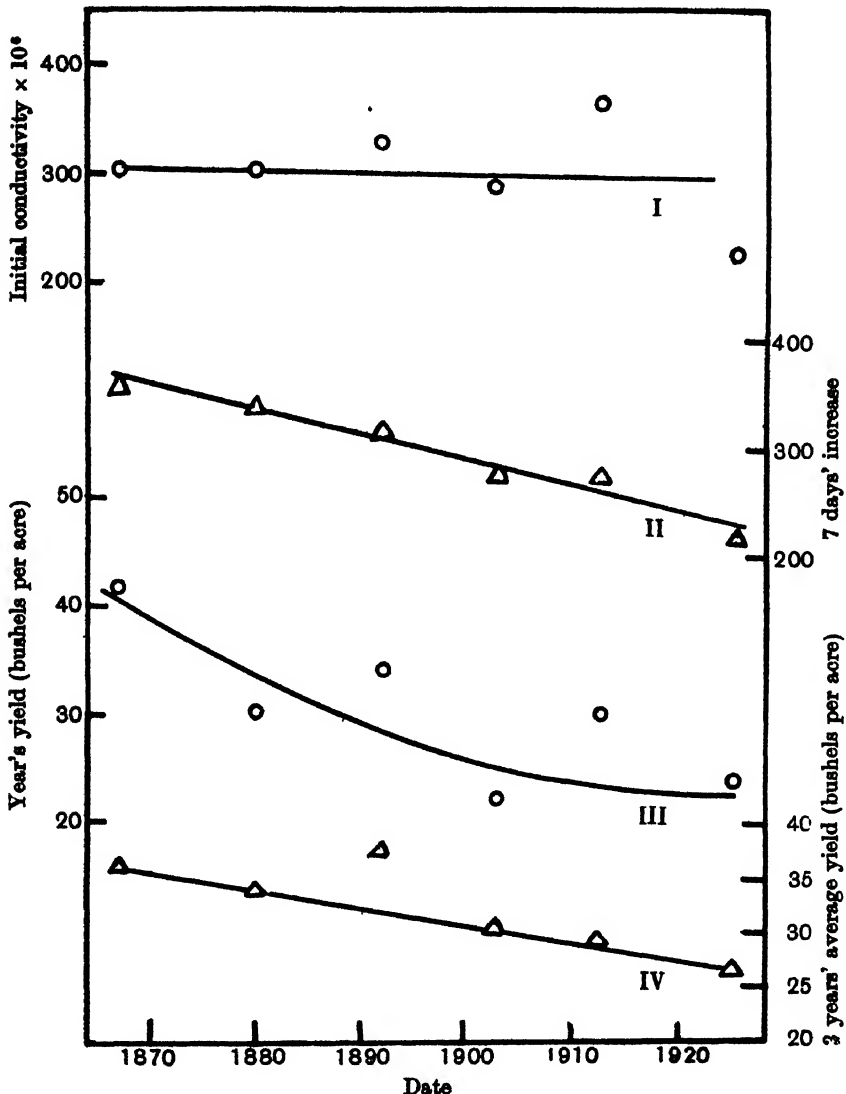


Fig. 5. Broadbalk Plot 2B. Farmyard manure. For explanation of numbering see Fig. 2.

from Plots 2B and 7 (1868 and 1881 respectively) are lower than the earliest samples from the unmanured plot (1846 and 1856). Since all the plots received exactly the same treatment prior to 1846, it is reasonable to conclude that, in spite of the manures applied, the normal conduc-

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tivities fell between 1846 and 1869 in the case of Plot 2B and between 1846 and 1881 in the case of Plot 7. After this date the conductivities remained fairly constant for any one plot. It is also of interest to note that this constant value for plots 2B, 7 and 3 are in descending order of magnitude. It appears, therefore, from the foregoing results that, under continuous cropping, the normal initial conductivity of a soil, whether uniformly manured or unmanured, decreases to a low value, which afterwards remains or tends to remain fairly constant over a large number of years, the minimum value depending slightly on the kind and probably on the quantity of manure applied.

### BROADBALK WILDERNESS AND ADJOINING GRASS LAND.

That fertility increases when land is in the state of permanent grass or under natural vegetation is well known. The purpose of the present experiment was to see if such gain in the fertility is reflected in the measurements. In 1882 a portion of the upper end of Broadbalk field was not harvested, but was fenced and allowed to run wild. A more detailed description of this area, known as Broadbalk Wilderness, together with figures for nitrogen that accumulated in 20 years showing increased fertility, is given by Hall<sup>(4)</sup>. There is also a strip of land between the lower end of the Broadbalk plots and the brick trench for collecting pipe drainage, which has been under a thick growth of grass for many years. In 1928 separate samples were taken from the portions of the Wilderness and of the grassland which was previously included in Plot 3. Measurements were made on these samples after they had been dried in air. The results, together with the measurements for Broadbalk Plots 3 and 2B for comparison, are given in Table I below.

Table I.

Plot no.	Sample collected in	Initial conductivity $\times 10^6$	7 days' increase $\times 10^6$
3	1881	197	157
Wilderness	1928	338	295
3	1894	179	136
Grassland	1928	277	223
2B*	—	305	293

\* Mean values of all the samples between 1868–1927.

If the soil conditions of the Broadbalk Wilderness in 1882 and those of the grassland in 1896 are assumed to be very nearly the same as those of Plot 3 in 1881 and 1894 respectively, the above results show that



the increased fertility which the Wilderness and the grassland have gained by 1928 was reflected also in the measurements. It is interesting also to find that the mean measurements for Broadbalk Plot 2B approximate closely to the measurements for the Wilderness and the grassland. This shows that by yearly application of dung the fertility of the soil can be maintained near that of a soil under natural vegetation.

#### RELATION OF 7 DAYS' INCREASE TO YIELD OF CROP.

It has been shown that under continuous cropping the 7 days' increase decreases steadily, and also the crop yield, and that there is a distinct parallelism between them. The significantly high positive correlation coefficient between the two curves, II and III in Fig. 2 (+0.859), is undoubtedly the best indication of the parallelism between the two series of values. When, however, the time factor is eliminated from the two series, the partial correlation coefficient between them is found to be insignificant, viz. +0.061. The result is not surprising, since the variation in the crop yield is the resultant of three types of variation as distinguished by Fisher(3); these are (i) annual variation, (ii) steady diminution due to deterioration of the soils, and (iii) slow changes other than steady diminution. The main trend of the curve for crop yield is due to the progressive changes taking place in the soil, whereas the annual fluctuations are doubtless due to climatic factors both in the case of wheat(3) and barley(5). Evidence has been obtained by the senior author(7) that these climatic factors have little effect on the 7 days' increase of arable Rothamsted soils, and consequently a high partial correlation between this measurement and crop yield would not be expected. On the other hand, the high positive correlation obtained between the two quantities shows that the 7 days' increase is a very satisfactory indication of the changes taking place in the fertility of the soil itself.

The results obtained in this paper, therefore, lead to the conclusion that, under continuous cropping, there is a steady diminution, at first rapid but afterwards slow, in the 7 days' increase of a soil, particularly if the soil is unmanured, and that this gradual diminution is correlated with a gradual fall in the crop yield.

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### SUMMARY.

Measurements have been made of the electrical conductivity of aqueous suspensions of stored soil samples taken at intervals of several years from four of the Rothamsted classical plots bearing the same crop every year. The results show that under continuous cropping:

1. The initial conductivity (and therefore the soluble salt content) of an unmanured soil decreases steadily to a minimum value, which then remains fairly constant over a long period of years. There is reason to believe that, under the same conditions, the normal initial conductivity of a continuously manured plot (excluding the temporary increased measurements due to application of manure) decreases similarly to a fairly constant minimum value, which is slightly greater than that of an adjacent unmanured soil depending on the kind and probably on the quantity of manure applied.

2. The 7 days' increase of both unmanured and manured soil decreases progressively. In the case of an unmanured soil for which earlier samples were available, the 7 days' increase is found to decrease comparatively rapidly during the first few years of continuous cropping.

3. The 7 days' increase of a soil manured every year is maintained throughout at a higher level than that of an adjacent unmanured soil. The difference in the levels is greater when dung is applied than when inorganic fertilisers only are used.

4. There is a high positive correlation (+0.859) between the 7 days' increase of stored samples for various years and the crop yield for those years. But when a partial correlation is calculated, eliminating the time factor, the value is found to be insignificant (+0.061).

Evidence is also given that, on allowing a soil in a low state of fertility due to continued cropping to run wild, or on leaving it under grass, there is a marked increase both in its initial conductivity and 7 days' increase. This result is thus in accordance with the well-known fact that a soil left to either of the above two conditions gains in fertility.

During a preliminary investigation it was observed that, on prolonged storage of an air-dry soil, the initial conductivity is not altered significantly, but the 7 days' increase rises rapidly in the course of a few months to a fairly constant maximum value. This value, and the time required to attain it, both depend on the previous manurial treatment of the soil.

## ACKNOWLEDGMENTS.

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# THE COLORIMETRIC DETERMINATION OF PHOSPHORIC ACID IN HYDROCHLORIC ACID AND CITRIC ACID EXTRACTS OF SOILS.

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MACROMETHODS for the determination of acid soluble phosphoric acid in soils depending on its precipitation as ammonium phosphomolybdate suffer two disadvantages. In the first place the analytical operations—evaporation, ignition and quantitative filtration—are such that the time and lay-out involved are excessive. Secondly, the determinations sometimes show bad duplication. In some instances the precipitation is the source of error, while in others the ignition of the residue prior to the acid extraction of the phosphoric acid is the critical stage. Further, iron salts and the use of an ammonium molybdate solution, which has become insensitive through ageing, may prevent complete precipitation. For these reasons colorimetric methods should have the advantages not only of reducing the time of working, but of giving more accurate determinations for small amounts of phosphoric acid in acid extracts of soils.

I. Lonstein<sup>(1)</sup> applied the method of Denigès<sup>(2)</sup>, which has been widely used for water extracts, to hydrochloric acid and citric acid extracts of a number of South African soils, and obtained good agreement with the gravimetric method. Briefly the treatment prior to developing the colour consisted of evaporating a small volume of the extract to dryness after the addition of calcium acetate solution. The residue was ignited to destroy the organic matter and to render the silica insoluble, and was then extracted with 10 per cent. sulphuric acid. After filtration excess acid was removed with ammonia and the colour developed with the ammonium molybdate and stannous chloride solutions.

Table I gives the results obtained by us for a number of English soils in a comparison of the gravimetric determinations as phosphomolybdic anhydride with colorimetric ones on extracts prepared by Lonstein's method, and using both the Denigès and Fiske-Subbarow reagents<sup>(3)</sup> to develop the colour.

These figures reveal the presence of some disturbing factor in the analysis of the heavier soils especially when the Denigès method of



colour production was used. The asterisk indicates that the blue colour developed slowly and had a green tint.

Table I.

Soil No.	Geological formation	$P_2O_5$ per 100 gm. of soil					
		Hydrochloric acid extract			Citric acid extract		
		Gravi-metric	Denigès	Fiske-Subbarow	Gravi-metric	Denigès	Fiske-Subbarow
A. 1262	Greensand	0.170	0.166	0.168	0.0501	0.048	0.049
		—	0.170	0.167	—	0.049	0.049
		—	0.165	0.168	—	—	—
A. 1263	Millstone Grit	0.076	0.069	0.073	0.0119	0.0117	0.0120
		—	0.071	0.074	—	0.0117	0.0119
		—	0.073	0.074	—	—	—
A. 1264	Red Clay loam over Lower Oolite	0.236	0.210*	0.221	0.0034	0.001*	0.0021*
		—	0.205*	0.216*	—	0.002*	0.0027*
		—	0.201*	0.209*	—	—	0.0015*
S. 761b	Clay loam with flints	0.132	0.117*	0.127	0.0124	0.008*	0.009*
		—	0.122	0.121*	—	—	0.008*
		—	0.120	0.124	—	0.005*	0.010*
S. 762	Clay loam with flints	0.195	0.175*	0.179*	0.0522	0.041*	0.045*
		—	0.170*	0.183	—	0.046*	0.047*
		—	0.183	0.182	—	0.040*	0.047*

In the analyses for which no results are reported in this table the green tint had become so pronounced that it was impossible to make comparisons.

Subsequently it was found that ferric salts added to a pure phosphate solution retarded and reduced the colour development, and gave the same green tint and fading that was observed during the analyses of the above soils. Ferrous salts had little effect. Further, the sulphuric acid extracts from the clay soils contained appreciable amounts of iron, while those from the lighter soils had only traces. This was regarded as additional evidence for the view that ferric iron was the interfering constituent. Attempts to reduce the amount of soluble iron by varying the time and temperature of ignition (using an electric muffle furnace) and the time of extraction with acid failed to give consistent results. While Matther(4) makes no mention of this difficulty when using the method of Fiske-Subbarow, Denigès(5) in a later paper overcame the interfering action of ferric salts by the use of metallic copper for reducing the iron and the ammonium phosphomolybdate. Belgrave(6) was unable to obtain satisfactory results by this procedure on Malayan soils, but obtained satisfactory results when zinc was used for the reduction of the iron and the colour developed in a solution of carefully controlled acidity.

Accurate colorimetric determination of phosphoric acid in soil extracts, therefore, demands not only the absence of large amounts of silica and organic matter and a controlled acidity, but also the absence of ferric iron. To satisfy these conditions a method was devised in which the organic matter, including citric acid, was oxidised by sodium permanganate in hydrochloric acid solution, the ferric iron precipitated by potassium ferrocyanide and the acidity adjusted by neutralisation with ammonia. As far as possible in this method lengthy operations such as quantitative filtration, dilution, evaporation and ignition were eliminated or reduced to a minimum. For this reason sodium permanganate with its higher solubility was used instead of the potassium salt for the wet oxidation process. The removal of any excess of oxidising substances, which was necessary for reasons discussed later, was found to be complete after a partial evaporation of the hydrochloric acid solution. In this way troublesome methods such as ignition or nitric acid treatment for the destruction of citric acid were avoided.

The procedure finally adopted for removing the iron was based on the following conclusions drawn from experiments on the behaviour of potassium ferrocyanide with iron, manganese, molybdenum, and aluminium salts when tested alone or in mixtures. Ferric ferrocyanide is very insoluble except in the presence of excess ferrocyanide. Manganese ferrocyanide is less insoluble than the ferric salt in acid solution but, at about the neutral point, it becomes so insoluble that the whole of the ferrocyanide can be removed in the presence of excess of manganese. Further, no manganese ferrocyanide is dissolved on subsequently acidifying the solution to about  $pH$  3.0. Molybdenum gives a brown coloration and precipitate with ferrocyanide. Thus, by ensuring an excess of manganese and carrying out the precipitation of the iron with potassium ferrocyanide in acid solution and subsequently bringing the reaction to neutrality, it is possible to remove both iron and ferrocyanide completely and, in addition, to prevent any discoloration when the molybdate solution is subsequently added. This adjustment to approximate neutrality is readily made by utilising the fact that, on progressive additions of ammonia, the ferrocyanide precipitate changes colour from blue to bright purple at  $pH$  6.8–6.9. It is, therefore, not necessary to add an indicator.

Since the phosphoric acid is also precipitated at this reaction, it becomes necessary to add sufficient acid to redissolve it. An upper limit to the amount permissible is set by the sensitivity of the colorimetric methods, especially Denigès, to acidity and the possibility of redissolving

the ferrocyanides. An acidity corresponding to pH slightly below 3 was found to be satisfactory, and can be reached most conveniently by adding a fixed amount of acid to the mixture so long as constant volumes of soil extracts and reagents are used. Spot tests with bromo-phenol blue can be used as a check or as an alternative.

With 1 per cent. citric acid solutions to which some iron and varying amounts of phosphoric acid ( $\text{KH}_2\text{PO}_4$ ) had been added, the above treatment gave 99.3 per cent. recovery for 0.01–0.05 mgm.  $\text{P}_2\text{O}_5$  by the Denigès method and 99.6 per cent. for 0.3–1.5 mgm. by the Fiske-Subbarow procedure.

The work of Parker and Fudge(7) on the effect of silica on the Denigès method and of Fiske and Subbarow on their own method suggested that interference from this source would not occur with citric acid extracts of soils. Confirmation of this was obtained from parallel tests with and without the removal of silica. In the case of hydrochloric acid extracts, however, the dissolved silica may amount to 0.2–0.9 per cent. and since in order to obtain a convenient depth of blue colour the volume of iron-free solution taken for colour development will vary with the concentration of phosphoric acid, it follows that interference by silica may occur with soils low in hydrochloric acid soluble phosphoric acid. Table II however shows that the effect is small except for soil S. 811b, where a value 20 per cent. higher is obtained when silica is not removed. Nevertheless the absolute error is small. It is therefore recommended that for soils with less than 0.02 per cent.  $\text{P}_2\text{O}_5$  soluble in hydrochloric acid, complete evaporation to dryness and resolution with hydrochloric acid be substituted for the partial evaporation after the addition of sodium permanganate.

Table II.  $\text{P}_2\text{O}_5$  extracted by hydrochloric acid per 100 gm. of soil.

Soil	Denigès method		Fiske-Subbarow method	
	$\text{SiO}_2$ removed	$\text{SiO}_2$ not removed	$\text{SiO}_2$ removed	$\text{SiO}_2$ not removed
S.	0.123	0.124	0.123	0.123
S. 805	0.191	0.193	0.190	0.191
S. 811a	0.030	0.030	0.030	0.030
S. 811b	0.011	0.013	—	—

The results of analyses in Table III of a variety of soils by the method finally adopted show very satisfactory agreement with the gravimetric method.

*Determination of Phosphoric Acid*

Table III.

Soil No.	Description	% $P_2O_5$ (HCl soluble) Method			% $P_2O_5$ (citric soluble) Method		
		Gravimetric	Denigès	Fiske-Subbarow	Gravimetric	Denigès	Fiske-Subbarow
A. 1262	Greensand	0.170	0.168	0.169	0.050	0.049	0.050
A. 1188	Moorland	0.049	0.048	0.050	0.0044	0.0042	0.0044
A. 1263	Millstone Grit	0.076	0.077	0.076	0.0119	0.0118	0.0119
S. 524a	Sandy loam	0.131	0.133	0.130	0.0210	0.0205	0.0205
S. 524b	Sandy loam	0.253	0.249	0.250	0.081	0.080	0.080
—	Fen	0.177	0.174	0.176	0.041	0.039	0.042
A. 1264	Red Clay loam (over Lower Oolite)	0.236	0.239	0.234	0.0037	0.0035	0.0036
S. 503	(Red Clay loam (over Lower Oolite)	0.124	0.126	0.123	0.0088	0.0085	0.0089
S. 497	Red Clay loam (over Lower Oolite)	0.102	0.101	0.099	0.0041	0.0042	0.0040
A. 1265	Clay with flints	0.087	0.085	0.086	0.0043	0.0045	0.0043
S. 761b	Clay with flints	0.132	0.130	0.131	0.0124	0.0120	0.0121
S. 762	Clay with flints	0.195	0.191	0.192	0.052	0.051	0.051
S. 761a	Clay with flints	0.247	0.243	0.245	0.062	0.063	0.061
—	Gold Coast	0.0135	0.0139*	0.0136*	—	—	—
—	Borneo	0.0066	0.0069*	—	0.00024	0.00026	—

\*  $SiO_2$  removed.

These methods are being applied to the determination of total and citric acid soluble phosphoric acid in basic slags. Table IV illustrates their value for the rapid analysis of phosphatic manures.

Table IV. %  $P_2O_5$  total.

Method	Mineral phosphate	Basic slag		Super-phosphate
Gravimetric (pyrophosphate)	No. p. 119 25.92	No. p. 120 14.92	No. p. 121 15.10	No. p. 122 16.06
Colorimetric (Fiske-Subbarow)	25.7	14.4	15.0	16.1

It is believed that the low result for No. p. 120 is due to the interfering action of vanadium which produces a yellow colour in the solution by reaction with the potassium ferrocyanide.

In this work a Klett-Kober colorimeter was used, and the comparison was considered valid only if the colour ratio did not exceed 1.3. With a little experience of the Denigès or Fiske-Subbarow method the appropriate standard can be made from an inspection of the test 2 to 3 minutes after the reducing solution has been added. The colours of test solutions prepared by the procedure given in this paper develop at the same rate as the standards, whereas for those obtained by the ordinary method the rates are variable and always slower, even where the amount of iron was insufficient to affect the result. In consequence the selection of the

correct standard can now be made quite easily and quickly, and a dozen tests compared in half an hour from the time the solutions are developed.

Where a colorimeter is not available, Nessler tubes, graduated and with stopcocks, may be used with somewhat reduced accuracy. When this method is adopted the test solutions must be adjusted to the weaker end of the colour range. A 25 c.c. Denigès standard and a 3-4 c.c. Fiske-Subbarow standard are the maximum depths of colour that it is possible to compare in this way.

The final methods adopted for the determination of phosphoric acid in hydrochloric acid and citric acid extracts of soils are given below.

#### HYDROCHLORIC ACID EXTRACTS. (Using the A.E.A. 1905 method.)

20 gm. of powdered soil (1 mm. sieve) are placed in a flask, covered with 70 c.c. of concentrated hydrochloric acid and boiled for a short time. The flask is loosely stoppered and the contents allowed to digest in the water-bath for 48 hours. The solution is then cooled, diluted and filtered. This filtrate is made up to 250 c.c.

#### *Destruction of organic matter and removal of iron.*

15 c.c. of the extract are pipetted into a 100 c.c. conical flask and 0.5 c.c. of 20 per cent. sodium permanganate (1) added. The flask is then placed on a hot sand bath in a fume cupboard for about 15 minutes. By this time the contents should be simmering and free from any brown manganese precipitate. The liquid is cooled and diluted to about 30 c.c., 6 c.c. of 10 per cent. potassium ferrocyanide (2) are added, followed by 5 c.c. of 10 per cent. manganese sulphate solution (3) with frequent shaking of the contents. After standing several minutes the mixture is titrated with ammonia (4) until the blue colour just turns purple. 3.5 c.c. of 2N sulphuric acid (5) are added and the whole transferred to a 100 c.c. graduated flask, diluted to the mark and filtered. The first few c.c. are discarded. Where many analyses are to be made this transference may be avoided by using a 100 c.c. graduated flask throughout. Of the filtrate aliquots are taken for colour development by the Fiske-Subbarow or Denigès method given below.

*Fiske-Subbarow.* 10 to 50 c.c. are pipetted into a 100 c.c. graduated flask, diluted to 75 c.c. approximately, 10 c.c. of ammonium molybdate (6) added, then 4 c.c. aminonaphthol sulphonic acid solution (7) and the liquid made to the mark. The flask should be shaken during each

addition. The contents are finally poured into a 100 c.c. conical flask. 15 minutes later the test is compared with a standard made similarly from the standard phosphate solution (8).

*Denigès.* 1 to 25 c.c. are pipetted into a 100 c.c. graduated flask diluted to 90 c.c. and 1 c.c. ammonium molybdate (9) and three drops of stannous chloride solution (10) added, the flask being shaken between each addition. After diluting to the mark the contents are poured into a 100 c.c. conical flask, and compared after 5 minutes with a standard made similarly from the standard phosphate solution (11).

#### CITRIC ACID EXTRACTS.

25 gm. of soil (through 2 mm. sieve) are placed in a half-litre bottle, and 250 c.c. of 1 per cent. citric solution added with extra citric acid equivalent to the calcium carbonate present and shaken in a mechanical shaker for 24 hours. The solution is then filtered. If the first portion of the filtrate is not clear it is returned to the filter.

#### *Determination of organic matter and removal of iron.*

75 c.c. are pipetted into a 300 c.c. Kjeldahl flask, 10 c.c. of concentrated hydrochloric acid added and followed by 12 c.c. 20 per cent. sodium permanganate (1). The sides of the flask are washed down with a little water. After standing half an hour the contents are vigorously digested till no manganese precipitate remains (about  $\frac{1}{2}$  hr. more). The contents are transferred with a minimum of water to a 100 c.c. graduated flask. 4 c.c. 10 per cent. potassium ferrocyanide (2) are added slowly, drop by drop, with frequent shaking. Several minutes later the mixture is titrated with ammonia (4) until the blue colour just turns purple. 1.5 c.c. 2N sulphuric acid (5) are then added and made to the mark with water. After the solution has been filtered and the first few c.c. discarded, the colour is developed in an aliquot by one of the methods given under the analysis of hydrochloric acid extracts.

Where additional citric acid has been added for soils containing calcium carbonate, the amount of sodium permanganate should be increased proportionally.

#### REAGENTS.

(1) 20 per cent. sodium permanganate solution. 200 gm. of pure sodium permanganate are dissolved to 1 litre.

(2) 10 per cent. potassium ferrocyanide solution.

(3) 10 per cent. manganese sulphate solution. 50 gm. of  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$  (A.R.) are dissolved to 500 c.c.

(4) *Ammonia* 1·1. 1 vol. of 0·880 ammonia (A.R.) is diluted with an equal volume of water.

(5) *2N sulphuric acid*.

(6) *Ammonium molybdate reagent*. (Dissolve 25 gm. of ammonium molybdate (A.R.) in 200 c.c. of water.) Rinse into a 1 litre graduated flask containing 500 c.c. of 1N sulphuric acid. Dilute to the mark.

(7) *Aminonaphthol sulphonic acid reagent*. Dissolve 0·5 gm. of aminonaphthol sulphonic acid 1:2:4 in 195 c.c. of 15 per cent. sodium bisulphite. Add 5 c.c. of 20 per cent. sodium sulphite and then further lots of 1 c.c. with shaking until solution is complete. This reagent will keep for a week in a stoppered bottle.

(8) *Standard phosphate solution*. Dissolve 0·1917 gm.  $\text{KH}_2\text{PO}_4$  (A.R.) in 1 litre.

$$1 \text{ c.c.} = 0\cdot0001 \text{ gm. } \text{P}_2\text{O}_5.$$

Addition of toluene to the solution is recommended.

(9) *Ammonium molybdate reagent*. Add 100 c.c. of 10 per cent. ammonium molybdate solution to a mixture of 150 c.c. water and 150 c.c. concentrated sulphuric acid (A.R. and arsenic free). The reagent should be kept in the dark.

(10) *Stannous chloride solution*. To 0·1 gm. of tin foil add one drop of a 4 per cent. copper sulphate solution and 2 c.c. of concentrated HCl. When reaction is complete dilute to 10 c.c. and filter. The reagent must be prepared freshly each day.

(11) *Standard phosphate solution*. Dissolve 0·1917 gm.  $\text{KH}_2\text{PO}_4$  (A.R.) in water and dilute to 1 litre. Take 10 c.c. and again dilute to 1 litre.

$$1 \text{ c.c.} = 0\cdot000001 \text{ gm. } \text{P}_2\text{O}_5.$$

Addition of toluene to the solution is recommended.

#### NOTES.

1. For peaty soils 2 c.c. of 20 per cent. sodium permanganate should be used for the oxidation of the hydrochloric extract.

2. For soils with less than 0·02 per cent.  $\text{P}_2\text{O}_5$  soluble in hydrochloric acid the solution after oxidation should be evaporated to dryness, taken up with 2 c.c. concentrated hydrochloric acid and water and the iron then precipitated without filtering off the silica.

3. Ferricyanides must not be present, as the solution after the precipitation of the iron will be coloured. Oxidising substances, therefore, must also be absent.

4. The addition of the stated amounts of 10 per cent. sulphuric acid to the mixture of ferrocyanides produces an acidity neither excessive, after taking aliquots and diluting, for colour development nor too low for solution of the phosphate. If necessary one drop may be withdrawn, and a spot test on a filter paper with bromo-phenol blue should give a full yellow colour.

The same drop will also serve for a ferric iron test.

5. The range of standards for Fiske-Subbarow method is 2 to 15 cm. of the standard phosphate solution and that for Denigès is 5 to 50 c.c. For colorimeter work the deeper colours are preferable.

6. The factor 0.995 was used in the results given to correct for the volume of the ferrocyanide precipitate.

#### SUMMARY.

1. The colorimetric determination of phosphoric acid in hydrochloric and citric acid extracts of soils by a method involving the evaporation of the extract, ignition and acid extraction of the residue with either the Denigès and Fiske-Subbarow methods of colour development was satisfactory only with light soils.

Clay soils gave low results owing to the presence of larger amounts of iron.

2. A method is given in which the organic matter and iron are removed by treatment with sodium permanganate and potassium ferrocyanide. The results are in good agreement with the gravimetric method.

#### ACKNOWLEDGMENT.

One of us (A. J. P.) desires to thank the Ministry of Agriculture for a Scholarship, which enabled him to co-operate in this work.

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## THE USE OF HYDROGEN PEROXIDE FOR ESTIMATING HUMIFICATION

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The work described in this note was carried out some years ago, in order to estimate the value of the 6 per cent hydrogen peroxide method for determining "degree of humification" in soils or decomposed organic materials. Clearly if a method is to effect even "an approximate separation between humified and unhumified matter" (4) the reagent used should have the minimum of action on unhumified material. In the original description of the method (4) it was shown that cellulose and "crude fiber" from hay, straw, and wood were negligibly attacked, but that washed hay and straw suffered appreciable loss (17.8 and 11.5 per cent respectively). Since in point of fact it is not "crude fiber" but whole plant material that is decomposed in the formation of humus, it was thought necessary to have more information concerning the extent to which non-humified plant materials are affected by the 6 per cent peroxide reagent before it was used for comparing the humification of materials of different origin. The results were so discouraging to the adoption of the method for quantitative purposes that the work was carried no further.

Recently, however, in a critical study of methods for determining the "humified" portion of the soil organic matter, Waksman and Stevens (5) examined the 6 per cent peroxide method along with others in studying in considerable detail four materials—sound wood (finely ground), rotted wood, forest soil, and lowmoor peat soil. After comparing the action of hydrogen peroxide and chlorine dioxide on certain fractions of these substances, those authors concluded that results with oxidizing agents could not be interpreted in terms of definite constituents of the plant residues, and so could not be used in determining degree of decomposition. They further commented on the extent (20 per cent) to which even sound wood was oxidized by the peroxide reagent. As an extension of this observation, it seemed useful to put on record the results of the present author's work: here the action of peroxide on a number of fresh plant materials had been examined, along with the extent to which it might be attributed to simple solvent action, while the further influence of soil on peroxide action was made clear.

It was found that the action of 6 per cent peroxide on all the plant materials examined was considerable, varying from 19 per cent with straw to 61 per cent with mustard tops; the effect was increased in every case by mixing with soil,

with values then ranging from 21 per cent (straw) to 70 per cent (sphagnum). With most materials, however, a great part of the effect of the peroxide reagent was simply solvent action—that is to say, treatment with water alone under the conditions of the method caused considerable losses, and the extracted residues were more resistant to peroxide. With two exceptions (sphagnum, 49 per cent, and mustard, 31 per cent) none of the residues after water extraction lost more than 16 per cent of their weight under peroxide treatment.

This result might supply some justification for the use of the peroxide method—so far as its action on undecomposed material is concerned—if it were possible to assume that under natural conditions the water-soluble portions of plant materials were completely leached away by rain and played no part in “humification.” But this is unlikely, and in any case the catalytic influence of the inorganic matter, in soils, would increase the action of peroxide on the insoluble plant material. Consequently, from the point of view of the action of the peroxide reagent on *undecomposed* plant material, estimates of “degree of humification” by this method can only be very approximate; and with some materials, particularly those including sphagnum, quite unreliable.

From the point of view of the action on *decomposed* plant material, the adverse verdict of Waksman and Stevens has already been noted. In a paper of which only the abstract is at present available McLean (3) examined the action of hydrogen peroxide in a different way, by studying the effect of varying concentrations on organic matter in the soil. Regular variations in the attack of peroxide on soil nitrogen and carbon were found indicating an optimum concentration of peroxide (3 per cent), at which soil organic matter was differentiated into “two categories whereby it is possible to arrive at the condition of the organic matter of a soil at any particular time.” The relationship of these categories to the degree of humification of the organic matter does not seem to be sharply defined; in any case if the 3 per cent reagent were to be adopted for this determination it would seem desirable first to examine its action on a variety of undecomposed plant materials.

In discussing the use of 6 per cent peroxide for estimating degree of humification it should not be overlooked that in practice the method has given some degree of agreement with visual observations and the known history of samples (1, 2). It seems clear that decomposed substances are more attacked than fresh materials [compare also “fresh” and “rotted” wood (5)], whether the reason is largely a matter of changing chemical constitution or in part mechanical, a result of the disintegrated condition of the decomposed material. But quantitative significance cannot be attached to results obtained by the method.

#### EXPERIMENTAL

The procedure employed in the present work was based on that described by Glomme (1), working with forest soils.

Two grams of oven-dried material was treated in a 250-cc. beaker with 60 cc. of 6 per cent hydrogen peroxide, heated on a water bath for 15 minutes, brought

to the boiling point over a flame, and filtered while hot through a fluted filter paper. The residue was completely washed out of the beaker with hot peroxide, and further washed on the filter with boiling peroxide until visible reaction (production of bubbles) ceased. Then after being washed three or four times with boiling water, it was transferred to a tarred porcelain or silica dish, dried in an oven for about 15 hours, weighed, ignited at red heat, and again weighed. In this way it was possible to estimate both "peroxide loss" and loss on ignition.

In the first series of experiments, samples of various plant materials were treated by this "standard method." In a parallel series, the materials were mixed with soil: 2 gm. of a mixture containing 20 per cent of added plant

TABLE 1  
*Action of hydrogen peroxide on unhumified plant materials*

	PEROXIDE LOSS	PEROXIDE LOSS IN PRESENCE OF SOIL	SOLUBLE IN BOILING WATER	PEROXIDE LOSS AFTER EXTRACTION	TOTAL LOSS, BOILING WATER FOLLOWED BY PEROXIDE
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Barley straw.....	16.8	21	11	8 [9]*	19
Hay.....	16.9	23	10	4 [5]	14
Larch needles.....	18.3	29	14	9 [10]	23
Beech leaves.....	21.6	27	15	8 [9]	23
Beech leaves, autumn.....	25.1	38	3	15 [15]	18
Pine needles.....	29.7	38	16	11 [13]	27
Lucerne tops.....	30.0	41	26	3 [4]	29
Oak leaves.....	30.5	38	21	5 [7]	26
Pasture grass.....	32.0	35	22	4 [5]	26
Oak leaves, autumn.....	34.8	44	13	14 [16]	27
Fungus mycelium.....	46.6	60	35	9 [14]	44
Sphagnum moss.....	48.3	70	5	46 [49]	51
Mangold leaves.....	48.3	67	41	5 [9]	46
Mustard tops.....	60.7	65	31	22 [31]	53

\* Figures in brackets are expressed as per cent of insoluble organic material.

To these values may be added those (already quoted) obtained by other investigators who examined undecomposed plant materials: "peroxide loss" of straw, 11.5 per cent; hay 17.8 per cent; finely ground chestnut wood 20.1 per cent.

material was used, and the loss in weight of the plant material was obtained by difference, the behavior of the soil alone under the standard method being first ascertained. In this series it was found that ordinary filter paper was weakened by hot peroxide in the presence of soil, and hardened paper (Whatman 50) was used. In another series, the "standard method" was followed except that distilled water was used throughout instead of hydrogen peroxide, the final volume of filtrate being brought to the same level as in the standard method. The residue, after being dried and weighed, was then treated with peroxide by the standard method.

The materials examined were taken as typical of those which play a consider-

able part in the production of humus in different kinds of soil; for forest soils, were chosen pine and larch needles, and the leaves of oak and beech (both green leaves and recently fallen autumn leaves); for arable soils, barley straw, man-gold leaves, and lucerne and mustard tops; for grassland, hay and young pasture grass were taken. Sphagnum moss was also examined, as being a major component of many peats; and the stems of the fructifications of a fungus, *Lepiota procera*, whose mycelium represents another material that contributes to "humus" formation. The samples were dried in a warm room, roughly ground in a hand mill, sieved (3 mm.), and oven dried for 20 hours. The soil used in one series of experiments was a Rothamsted (clay loam) soil poor in organic matter; it was similarly sieved and dried.

The results obtained are summarized in table 1. They are expressed as per cent of organic matter, taken as equal (for the purpose of this method) to the loss on ignition. The column "peroxide loss" expresses the loss in weight through treatment with 6 per cent hydrogen peroxide, which has been called the "degree of humification" in studies of decomposed materials. The values in this column are means of two or more determinations, which generally agreed closely; the greatest difference was under 2 per cent.

It is tempting to discuss these results in detail, especially in relation to what is known of the chemical composition of the different materials. High nitrogen substances after water extraction, for example, are particularly resistant, a result which may be compared with McLean's finding (3) of a resistant form of nitrogen in the soil organic matter. The measurements, however, are highly empirical; in addition to minor sources of inaccuracy, the results would be affected by changes in factors such as fineness of grinding or stage of growth of the material used. Their purpose was simple to supply information bearing on the use of the "6 per cent peroxide method," and from this point of view the results have been reviewed in the introduction.

#### SUMMARY

A number of undecomposed plant materials have been treated by the 6 per cent hydrogen peroxide method as used for estimating "degree of humification."

The action of the peroxide on these materials was far from negligible and was still greater in the presence of soil.

With the majority of the materials examined, simple solvent action was responsible for most of the loss under peroxide treatment, although there were notable exceptions.

From the point of view of its action on undecomposed materials the 6 per cent peroxide method can give only approximate results, and it is inadvisable to use it for comparing materials of widely differing origin.

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## XX. THE BIOLOGICAL OXIDATION OF CARBOHYDRATE SOLUTIONS.

### PART I. THE OXIDATION OF SUCROSE AND AMMONIA IN SECTIONAL PERCOLATING FILTERS.

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THE manufacture of sugar from the sugar beet gives rise to large volumes of waste water of a polluting nature. About five-sixths of the beet sugar factory effluent originates from the water which is used to wash and convey the beet from open storage silos or from trucks into the factory. Under usual conditions the conveying or fluming water requires only simple treatment so that it may be used again in the factory. The remaining one-sixth of the total effluent is obtained from the beet pulp, which after extraction of sugar by diffusion, is dried and used as cattle food. Before the spent pulp is dried, as much water as possible is squeezed out of it. A "press water" is thus obtained which is highly polluting by reason of dissolved organic substances. Numerous attempts have been made on the Continent to devise a process which would destroy the dissolved substances but the methods tried have not been attended with great success.

Press water is a liquid of varying composition, but to illustrate its nature the following analysis of a sample taken from a factory is given; sucrose 230, protein 23, pectin 4.3, pentosan 13.4 and saponin 0.2 parts per 100,000. In view of the pollution already experienced and the increase that would probably follow as a result of the extension of the beet sugar industry, investigations were begun in 1927 at the Rothamsted Experimental Station and at the Colwick factory of the Anglo-Scottish Beet Sugar Corporation, Nottingham, for the Department of Scientific and Industrial Research with the object of ascertaining whether liquids such as press water could be satisfactorily purified by biological oxidation on percolating filters. The results of the semi-commercial scale experiments at Colwick have already been briefly described in the first three annual reports of the Water Pollution Research Board of the Department of Scientific and Industrial Research [1928, 1929, 1930]. At Rothamsted preliminary experiments showed that effective oxidation of solutions of the approximate composition of press water could be achieved, after dilution, by filtration through biological or percolating filters. The filters consisted of earthenware drainpipes 24 inches deep and 6 inches diameter filled with 1/8 to 1/4 inch clinker. In order to obtain artificial press water, sliced mangolds

or sugar beet were first boiled with water, the sugar extract strained off, and the residual pulp was then squeezed in a press. The runnings were diluted to the desired strength. Such artificial press liquors were most effectively oxidised when diluted so as to contain 0.1 % of sucrose, and at this strength they were allowed to trickle continuously on to the filters at a rate of 4.6 cc. per minute, equivalent to 100 gallons per cubic yard of filter per day. In this manner 90-95 % of the sugar was oxidised.

It was observed that the mangold or beet extracts rapidly became acid, owing to the fermentation of the sugar. Thus the  $p_H$  value of the freshly made solutions fell from 7.5-8.0 to 3.0-5.0 in less than 24 hours. The effluent obtained from the filtration of such an acid solution, however, was invariably neutral or slightly alkaline. When the acid fermented solutions were made alkaline with milk of lime and then filtered, the activity of the micro-flora seemed to be impaired in some way and an effluent of inferior quality resulted. In later experiments it was found that when a solution made from beet press water was fermented for 8 hours at 35°, the products were approximately 45 % unchanged sugar, 40 % hydroxy-acids and 15 % aliphatic acids. The behaviour on filtration of solutions composed of sugar and such typical products of fermentation as acetic and lactic acid was therefore studied in some detail. The results of these experiments proved conclusively that the course taken by natural fermentation provides a suitable liquid for oxidation on percolating filters; a mixture of sugar, acetic and lactic acids derived from a fermented solution of sugar is more easily oxidised than the original sugar solution. Only a partial conversion of sugar to acid is desirable. It seems that the acids alone do not provide the filter organisms with the most suitable food material to encourage the growth of a highly efficient biological film; the acids are then only partially oxidised. Moreover, filters fed with either acetic or lactic acid require added nitrogen, whereas those provided with a mixed diet of sugar and acid appear able to utilise very small amounts of nitrogen or possibly to fix atmospheric nitrogen. An interesting observation made by Dr Sandon, of the Rothamsted Experimental Station, was that all the filters, whether fed with sugar, sugar plus acetic acid, sugar plus lactic acid, lactic acid, acetic acid or any similar combination with different forms of added nitrogen, seemed to support the same protozoan and insect population.

One of the most significant observations made during the 1928 experiments was the presence of nitrates in incompletely oxidised effluents. These experiments were carried out with the following solutions: (a) 0.1 % sugar with 0.003 % of nitrogen added as ammonium chloride, and made up in tap-water containing 0.0005 % of nitrogen as nitrate; (b) as (a) but with 0.003 % of nitrogen added as albumin. The effluents regularly contained 0.0015 % of nitrogen as nitrate when as much as 0.01 % of sugar was present at the same time. There was thus some evidence that nitrification may occur in presence of organic matter. This conflicts with the generally accepted view in sewage purification that oxidised nitrogen does not appear in an effluent until the

organic matter has been decomposed. Oxidation of carbon precedes that of nitrogen when solutions of organic nitrogenous compounds are stored in bottles and supplied with an ample quantity of oxygen. With asparagine, for instance, Adeney [1908] found that hydrolysis first takes place, giving aspartic acid and ammonia. This is followed by complete oxidation of the aspartic acid before the ammonia is nitrified. Although the results of previous investigations on the oxidation of dissolved substances have been confirmed, the generally accepted view that easily oxidisable carbonaceous matter inhibits nitrification on percolating filters was not supported by the results obtained with drainpipe filters. The frequent presence of nitrate and sugar in the laboratory filters might conceivably be explained by assuming that some of the sugar in the fresh liquor always side-tracked down the filter or ran straight through the medium and that such solution would form one of the numerous integral parts of the whole effluent. This possibility is dealt with in the present paper.

In some earlier experiments on biological filtration in 1927, mangold extracts containing 0.1 % of sucrose were run through drainpipe filters 2 feet deep. The disappearance of organic matter was measured at depths of 6, 12, 18 and 24 inches by means of the oxygen taken up by the sample from acid permanganate in 4 hours, with the result that the upper half of the filter removed the greater part of the organic matter just as in sewage filters. It is apparent that where the food supply is richest and the air supply is plentiful, micro-organisms will become more abundant. They are then able to utilise the strong solutions which are run on to them. Moreover, with liquids such as sewage, the bacteria and zoogaea can coagulate colloidal or suspended organic matter and then live on the coagulum. The lower part of a biological filter is thus apparently inefficient and contains less vital film only because its food supply is limited by the activity of the upper part.

The work described in the present paper was undertaken with the following objects:

- (1) to account for the advantage of oxidising a solution of sugar partly fermented to acids over that of an unchanged solution of sugar;
- (2) to follow the course of sugar decomposition and nitrification of ammonia through different levels of a percolating filter;
- (3) to ascertain the function of different sections of a percolating filter.

#### EXPERIMENTAL.

##### *Apparatus and methods of analysis.*

*Apparatus* (Fig. 1). The sectional filter used in these experiments consisted of 6 glass cylinders each 6 inches in diameter and 9 inches deep. The cylinders rested in perforated zinc trays supported on the ledges of a vertical frame. Liquid passing through the perforations was caught on a funnel and then dropped on to the next section. In order to obtain a constant flow of sugar

solution through a tube wide enough to be cleaned easily of bacterial growths, the apparatus shown in Fig. 1 was used. This device controls the hydrostatic head of solution in an aspirator by means of a length of fine capillary tubing. The sections were filled with 1/8 to 1/4 inch clinker which had been well washed after use in similar filtration experiments.

**Methods of analysis. Sucrose.** (a) This was determined by the Hagedorn-Jensen method as modified by Hanes [1929]: 2.5 cc. of the solution were inverted with 1 cc.  $\text{NH}_2\text{SO}_4$  by heating on a boiling water-bath for 20 minutes and then neutralised with 1 cc. of  $\text{N NaOH}$ . The sucrose was returned as glucose. The limits of the Hanes modification are from 1.74 to 4340 mg. glucose per 100 cc.

(b) An alternative modification has been used for the range of 0.347 mg. to 3470 mg. glucose per 100 cc. A solution is made up containing 3.3 g. potassium ferricyanide and 10.6 g. sodium carbonate per litre. 10 cc. of this solution and from 0.1 to 10 cc. of the unknown solution are heated according to the usual procedure and the residual ferricyanide is finally determined by reducing it with KI and titrating with  $\text{N}/200 \text{ Na}_2\text{S}_2\text{O}_3$ .

**Invert sugar.** The Hagedorn-Jensen solutions were used but 10 cc. of the ferricyanide solution were taken instead of 2 cc. and the volume of invert sugar made up to 10 cc. The range of the method is thus greatly increased, the difference between the blanks on successive days is also reduced and more reliable results are obtained than in the original Hagedorn-Jensen method. While the limits of the latter method are from 0.868 mg. to 347 mg. glucose per 100 cc. those of the modified method are from 0.174 mg. to 1735 mg. per 100 cc.

**Nitrogen as  $\text{NH}_3$ .** (a) Whenever the volume of solution permitted 250 cc. were distilled with ammonia- and nitrogen-free  $\text{NaOH}$  made according to the Ministry of Health Methods of Analysis [1929, p. 20]. The distillate was titrated with  $\text{N}/25 \text{ H}_2\text{SO}_4$  using cochineal as indicator.

(b) When the above method was impracticable 50 cc. of the solution were evaporated to dryness in a water-bath and the residue was taken up in 5 cc.  $\text{N NaOH}$  and 50 cc. 95 % alcohol. This solution was then steam-distilled in Foreman's apparatus [1928] until 100 cc. of distillate were collected and the ammonia was titrated with  $\text{N}/25 \text{ H}_2\text{SO}_4$ . It was found that this method gave a complete recovery of ammonia from dilute solutions of ammonium salts.

**Nitrogen as  $\text{NO}_2$ .** This was determined colorimetrically as described in the Ministry of Health Methods [1929, p. 8].

**Nitrogen as  $\text{NO}_3$ .** The residue in the steam distillation flask left after the determination of ammonia by Foreman's method was neutralised with normal acid and made up to 200 cc. One-half of this solution was reduced by incu-

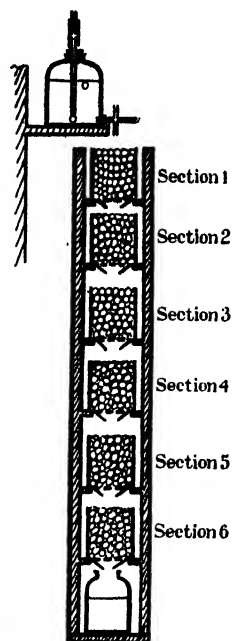


Fig. 1. Sectional percolating filter.

bation overnight at 35° with a zinc-copper couple; the ammonia was then distilled off and estimated by nesslerisation. The remaining half of the solution was incubated without the addition of a reducing agent, distilled and the distillate nesslerised. This represents the blank on the method. Details of the method are given in the Ministry of Health Methods [1929, p. 11].

*Oxygen absorbed from permanganate in 4 hours.* 10 cc. of  $N/8$   $KMnO_4$  and 10 cc. of 25 %  $H_2SO_4$  were incubated for 4 hours at 35° with sufficient solution of sugar or effluent to reduce not more than one-half of the permanganate. The unchanged permanganate was estimated with  $N/20$  sodium thiosulphate. The amount of permanganate reduced is practically proportional to the sugar in the solution, provided the quantity of permanganate decomposed remains constant.

*Biological oxygen demand, i.e. the oxygen absorbed from solution in 5 days or 20 days.* In order to carry out this determination the dissolved oxygen present in a mixture of tap-water and the solution under examination is found. The mixture is then incubated for either 5 or 20 days at 20° and the oxygen absorbed from solution in the oxidation of the organic matter is obtained from a second oxygen determination. Under rigidly controlled conditions the oxygen taken up is approximately proportional to the organic matter present in the diluted solution.

*p<sub>H</sub> value.* This was determined with the Hellige apparatus. The figures obtained gave good agreement when checked by the quinhydrone electrode method.

*Total nitrogen.* Before the solution was analysed as in the Kjeldahl method the nitrates and nitrites present were first reduced in the way described under *Nitrogen as NO<sub>3</sub>*.

## EXPERIMENTS.

*Exp. 1. Filtration of a mixture of sucrose, and acetic and lactic acids.* A solution composed of 0.033 % sucrose, 0.033 % acetic acid and 0.033 % lactic acid was made up in Harpenden tap-water and run on to the sectional filter at a rate of 9.0–9.5 cc. per minute. For the first 42 days the removal of organic matter from solution was measured only by the oxygen absorbed from permanganate and the biological oxygen demand tests. Adequate information on the rate of maturation of these filters had already been obtained and this information will shortly be published; hence no detailed analyses were made in the early stages of the experiment. The experiment proved that the acids were rapidly oxidised in the first and second sections. Thus the *p<sub>H</sub>* of the original mixture which was 4.65 increased to 6.0 in the first section and 6.5 in the second; it then increased uniformly until the liquid was discharged from the sixth section at a *p<sub>H</sub>* of 7.5. It should be noted that the nitric nitrogen in the final effluent during this period never exceeded 0.2 part per 100,000, although in the tap-water used to make up the nutrient solution there was 0.5 part of nitric nitrogen present.

*Exp. 2. Filtration of a solution of sucrose and ammonium chloride.* The further filtration of sucrose, and acetic and lactic acids was postponed on the 43rd day until suitable methods for the exact analysis of the substances in dilute solutions were available. From that day until the 88th day the following solution was fed to the filter at a rate of 9.5 cc. per minute.

Sucrose	...	...	...	1.0 g.
NH <sub>4</sub> Cl	...	...	...	0.1528 g. (increased on 120th day
K <sub>2</sub> HPO <sub>4</sub>	...	...	...	0.002 g. to 0.191 g.)
MgSO <sub>4</sub> ·7H <sub>2</sub> O	...	...	...	0.0006 g.
NaCl	...	...	...	0.0002 g.
CaCl <sub>2</sub> ·6H <sub>2</sub> O	...	...	...	0.1375 g.
Fe <sub>2</sub> Cl <sub>6</sub>	...	...	...	Trace
Distilled water	...	...	...	1 litre

C/N ratio until 120th day = 10/1: thereafter 8/1.

Analyses were made every few days over a period of 45 days and the results obtained are given in Table I.

Table I. *Percentage of sugar oxidised.*

Section	...	1	2	3	4	5	6
By 4 hours' test		22.5	39.0	46.0	53.2	58.4	67.7
By 5 days' test		25.2	42.6	44.4	50.2	56.5	65.5

The nutrient solution was made up daily and adjusted to a  $p_H$  value of 7.0.

As the filter began to mature a drop in  $p_H$  of the effluent from the first section indicated that acid was being produced in this part of the filter. For example, on the 58th day of the experiment the  $p_H$  figures were:

	Section					
Fresh liquid	1	2	3	4	5	6
7.0	6.6	6.9	7.3	7.3	7.4	7.4

Similar observations have previously been made in the Microbiology Department at Rothamsted.

The  $p_H$  figures given were obtained during about the first half hour, at the commencement of each day's run. Thereafter the  $p_H$  of the fresh liquid fell progressively during its 24 hours' storage. Even by careful sterilisation of the aspirator it was difficult to prevent the  $p_H$  of the nutrient solution from falling below 5.5 to 6.0 at the end of the day. As a rule, however, the aspirator was cleaned out daily with distilled water and every few days with hydrochloric acid. Under these conditions a liquid of  $p_H$  6.5 or less was filtered for the greater part of the day and there was no measurable production of acid. Typical examples are given in Table II.

Table II.  $p_H$  of nutrient solution and sectional effluent.

	Section					
Sucrose solution	1	2	3	4	5	6
6.5	6.8	7.3	7.4	7.4	7.4	7.4
5.5	6.6	6.9	7.0	7.1	7.2	7.2
4.6	6.4	6.8	7.0	7.1	7.2	7.2



*Nitrogen assimilation.* The nitrogen supplied as ammonium chloride was assimilated by the micro-organisms present in all the sections, but by those in No. 1 more than any other. It was evident from inspection that No. 1 section contained the bulkiest film. Compared with the other sections it oxidised the largest proportion of sugar. This is clearly shown in Table III.

Table III. *Nitrogen present in nutrient solution and effluents.*

Day of experiment	Nutrient solution	Section					
		1	2	3	4	5	6
54th	4.000	3.752	2.960	2.738	—	—	1.949
73rd	4.000	2.744	2.632	2.464	2.464	2.072	1.624
86th	5.000	4.704	3.472	—	3.248	—	2.688
112th	4.000	3.892	3.612	3.416	3.258	2.940	2.310
122nd	5.000	4.021	3.789	3.428	3.092	2.890	2.576
128th	5.000	4.372	4.230	—	—	—	—

It is apparent that nitrogen is continuously removed from the solution. This does not always signify an increase in vital film and therefore a correspondingly greater percentage oxidation of sugar; nitrogen may be lost by the decay of the organic film and its discharge as humus or by the removal of living micro-organisms in the final effluent or by the breeding and growth of flies on the filter, as rapidly as it is assimilated as ammonia.

*Nitrification.* In proportion to the amount of nitrogen locked up by the filter the quantity of nitrogen oxidised to nitrous and nitric acid is quite considerable. There appears to be a fine balance between the amount of nitrogen assimilated and carbohydrate oxidised on the one hand, and the amount of nitrogen oxidised. Where the film is thick and air passage somewhat restricted as in the upper sections, carbohydrate combustion is vigorous and nitrogen assimilation is appreciable. But in the lower sections, where organic matter is present in low concentrations (from 0.01 to 0.05 % sucrose) the filter is specialised for nitrification. It will be seen from Table IV, however, that

Table IV. *Nitrification in presence of ammonia and sucrose.*

Section	86th day Rate 9 cc. per min.				99th day Rate 5 cc. per min.				112th day Rate 5.85 cc. per min.			
	% sugar oxidised	N present as			% sugar oxidised	N present as			% sugar oxidised	N present as		
		NH <sub>3</sub>	NO <sub>2</sub>	NO <sub>3</sub>		NH <sub>3</sub>	NO <sub>2</sub>	NO <sub>3</sub>		NH <sub>3</sub>	NO <sub>2</sub>	NO <sub>3</sub>
1	9.4	4.66	0.01	0.03	10.8	5.0	0.01	0.0	37.6	3.89	0.05	0.17
2	9.7	3.43	0.01	0.03	24.0	4.3	0.01	0.12	4.4	3.61	0.05	0.20
3	6.7	—	0.01	0.03	5.3	4.2	0.02	0.24	6.3	3.42	0.05	0.29
4	8.7	3.17	0.01	0.07	28.6	3.0	—	—	9.9	3.26	0.06	0.32
5	10.7	2.08	0.01	0.15	6.6	2.5	0.03	0.25	8.3	2.94	0.07	0.37
6	22.0	2.57	0.02	0.10	—	—	—	0.52	11.6	2.31	0.05	0.61
Total	66.2				75.3				78.1			

complete absence of carbohydrate is not essential before nitrification can occur. On the 112th day, for instance, the effluent from Section No. 4 contained 44 parts of sucrose per 100,000, 0.06 part of nitrogen as nitrite and

0.32 part of nitrogen as nitrate. The solution passing through the filter receives a thorough mixing and it is therefore highly improbable that a small volume of nutrient solution passes through four sections and escapes oxidation while the rest of the solution undergoes a more complete oxidation. In other words, bacteria capable of oxidising ammonia to nitrite and nitrite to nitrate are actively present in a solution which contains free ammonia and carbohydrate.

*Exp. 3. Interchange of sections Nos. 1 and 2.* The effect of interchanging section No. 1 with No. 2 was studied from the 129th day of the experiment to the 157th. While section No. 2 showed a rapid increase in efficiency, due to the fact that it received a stronger nutrient solution, No. 1 fell off slowly. The net effect of the change, therefore, is to increase the capacity of the two upper sections to oxidise sugar. This is shown in Table V.

Table V. *Percentage of sugar oxidised.*

Section	Percentage of sugar oxidised.						
	Day before change	1st day of change	3rd day of change	6th day of change	14th day of change	20th day of change	29th day of change
No. 1	32.1	10.7	24.2	22.9	26.9	32.2	40.3
No. 2	8.0	34.4	35.9	24.4	21.4	17.0	26.8
No. 1 + No. 2	40.1	45.1	60.1	47.3	48.3	49.2	67.1
All sections	78	—	—	—	89.1	—	100.0
1 to 6	(about)						

It was shown in Exp. 2 that the production of acid in Section No. 1 depends on the  $p_H$  of the nutrient solution. If this is less than 6.7 or 6.8 then increase in acidity cannot be detected. The interchange of Sections Nos. 1 and 2 gave additional evidence in support of the view that the  $p_H$  of the nutrient solution controls acid formation. Thus in Table VI it is seen that for 2 days after Section No. 1 had been placed in No. 2 position, it received a liquid of  $p_H$  6.8. This section still retained its powers of acid production, for the  $p_H$  was lowered to 6.6. A few days after the change, however, it was unable to produce acid from a liquid of  $p_H$  6.8.

Table VI also indicates the rapid oxidation of acid, even by an immature section such as No. 2.

Table VI. *Effect of interchange on  $p_H$  values.*

	Day before change	1st day of change	2nd day of change	3rd day of change	6th day of change	14th day of change	29th day of change
Nutrient solution	6.0	6.3	6.8	6.0	4.4	6.0	6.6
Section No. 1	6.4	6.8	6.8	6.6	6.0	6.8	6.6
Section No. 2	6.4	6.6	6.6	6.8	6.4	6.9	6.8

Estimations of nitrate in the effluents of the sections interchanged were made at frequent intervals. These analyses are given in Table VII. It may be stated that the average amount of nitrate present in the effluent from the first section did not exceed 0.05 part N per 100,000, while in the second section this increased to 0.1 part. When the change of section was made nitrate was

definitely absent from the first effluent after the 6th day. The effluent from the next section (No. 1 temporarily in the place of No. 2) contained appreciable amounts of nitrate, though the results were somewhat erratic. However, sufficient nitrate was present to prove that nitrification had actually occurred in the presence of sucrose and invert sugar, equivalent in concentration to 0.06 to 0.07 % of glucose.

Table VII. *Nitrogen as nitrate.*

		Parts per 100,000.				
Day before change	Section	1st day of change	3rd day of change	6th day of change	14th day of change	29th day of change
Section No. 1 0.105	No. 2	0.095	0.056	0.024	0.0	0.0
Section No. 2 0.114	No. 1	0.125	0.111	0.048	0.378	0.200

*Exp. 4. Interchange of Sections Nos. 1 and 6.* In order to confirm the results obtained in Exp. 3, Section No. 6 was made the first section and No. 1 the last. This experiment brings out the effect of a 0.1 % sucrose solution on the biological activity of nitrifying organisms. It also demonstrates the reaction of a mixed biological flora to solutions of ammonia and nitrite in which little carbohydrate is present.

Turning to the consideration of the course of the sugar oxidation, it is seen that the oxidising power of Section No. 6 rapidly increased in its new position. Prior to the interchange the progressive removal of sucrose as the solution percolated through the filter was of the following order: Section No. 1, 40.3 %; No. 2, 26.8 %; No. 3, 6.8 %; No. 4, 11.8 %; No. 5, 12.0 %; No. 6, 2.3 %; total 100 %. When these figures are compared with those given in Table VIII, the development of active film in Section No. 6 is shown by the greater proportion of sugar removed by this section. Within one month it was as active as either Sections 1 or 2, when these received the fresh solution first.

Table VIII. *Percentage of sucrose oxidised.*

Day before change	Section	1st day of change	4th day of change	7th day of change	11th day of change	27th day of change
Section No. 1 40.3	No. 6 (now No. 1)	14.9	18.4	21.5	14.5	42.5
Section No. 6 2.3	No. 1 (now No. 6)	—	—	—	15.8	12.5

It is a remarkable fact that the filter, considered as a whole, did not suffer by the sudden change of conditions in this experiment. This is due partly to the fairly slow reduction in rate of oxidation shown by Section No. 1, partly to the efficiency of this latter section in the last position even after one month and partly to the increasing activity of Section No. 6 in the first position. That the overall efficiency of the filter was not impaired is seen from the following example of the percentage removal of sugar effected by the sections in the order they were present in the filter: Section No. 6, 42.5 %; No. 2, 13.9 %; No. 3, 14.9 %; No. 4, 9.0 %; No. 5, 1.7 %; No. 1, 12.5 %; total 94.5 %.

For the first few hours of the change of Sections Nos. 6 and 1, nitrite and nitrate were found to be present to the extent of 0.018 and 0.190 part N per 100,000 respectively in No. 6 effluent (temporarily No. 1); in No. 1 effluent (temporarily No. 6), the amounts were 0.036 and 0.230 respectively. Within one week nitrifying activity had almost ceased in No. 6, only 0.008 part of nitrogen as nitrite and 0.04 part as nitrate per 100,000 being obtained after 6 days. Section No. 1, however, became more active in this respect: the effluent contained 0.04 part of nitrogen as nitrite and 0.44 part as nitrate. There seemed to be a tendency for nitrate to appear suddenly in Section No. 6 and then disappear. Insufficient evidence exists on which to base any satisfactory explanation of this apparent anomaly.

*Inversion of sucrose during filtration.* When sucrose solutions were run on to non-sectional percolating filters only small amounts of invert sugar were found in the effluents. Analysis of samples taken at various levels of the sectional filter used in the previous experiments proved that a considerable fraction of the total sucrose was invariably changed into invert sugar. Typical results are given in Table IX.

Table IX. *Sucrose and invert sugar.*

Sample	Calculated as mg. glucose per 100 cc.							
	102nd day of experiment		122nd day of experiment		157th day of experiment		185th day of experiment	
	Sucrose	Invert sugar	Sucrose	Invert sugar	Sucrose	Invert sugar	Sucrose	Invert sugar
Fresh solution	108.02	1.10	112.34	0.86	130.9	—	111.50	—
Eff. No. 1	81.57	8.67	73.39	2.25	67.35	10.85	53.54	10.54
Eff. No. 2	68.04	9.88	56.88	4.83	33.28	9.72	37.90	10.75
Eff. No. 3	55.23	11.97	48.23	6.97	28.82	5.38	22.31	9.68
Eff. No. 4	37.66	15.26	36.15	11.15	10.44	8.26	15.26	6.73
Eff. No. 5	33.02	12.85	34.63	11.37	0.00	3.05	14.47	5.66
Eff. No. 6	35.25	11.75	32.11	11.69	0.00	—	4.07	2.34

It is seen from Table IX that appreciable amounts of invert sugar are present in certain sections of the filter. The quantity of invert sugar produced by any particular section does not remain even approximately constant during the development of the biological film. It is not improbable that the limiting factor in the oxidation of sucrose is the rate of oxidation of the invert sugar. Hence, when the filter was still immature (up to about the 130th day) invert sugar would tend to accumulate in the lower sections, owing to the inability of the organisms in these sections to decompose as much invert sugar as they produced. After the 130th day the filter reached maturity. The lower sections were then able to oxidise invert sugar *in situ* as rapidly as it was formed, in addition to part of that presented by the upper sections.

#### DISCUSSION.

The experiments described in this paper prove that a biological film may be built up on a filter made up of sterile clinker by allowing a 0.1 % solution of sucrose and an adequate supply of available nitrogen to percolate slowly

through the filter. If the rate of flow of this nutrient solution is kept low and aerobic conditions are maintained, any desired degree of oxidation can be effected. Before the microbial population has become established the clinker removes a considerable proportion of the sugar by adsorption but once the organisms begin to develop loss of sugar due to purely physical forces becomes insignificant. The sugar is then oxidised entirely by the micro-organisms. When the part played by each section of the filter used for these experiments is examined, it should be remembered that the first section is given an advantage in the initial stages which becomes more pronounced as the filter matures. This advantage lies in the concentration of sugar in the nutrient solution which is run on to No. 1 section. Table I exhibits the beginning of the superiority of Section No. 1 over all other sections. As this section oxidised 25 % of the sugar, the second section can only decompose 18 % of the total sugar, assuming an efficiency equal to that of the first section. Each development of film in the top section therefore increases the difference between the amount of sugar oxidised by Section No. 1 and by lower sections. Further advantages which the top section gains are due to the fact that the water-holding capacity of the film and its absorptive power increase with the growth of film. The sugar therefore remains in contact with micro-organisms for a longer period in this part of the filter than in any other. A quantitative expression of the average time of contact of liquids with filter medium has already been worked out by an elaboration of Clifford's method [1907] and it has been shown that the time of contact might increase to two or three times the contact of the clean, sterile medium, as a result of film development. The sectional filter was not regarded as mature until it was able to oxidise 90 to 100 % of the sugar it received. The percentage of sugar oxidised by the different sections was then approximately: No. 1, 40 %; No. 2, 25 %; No. 3, 12 %; No. 4, 10 %; No. 5, 7 %; No. 6, 6 %.

Under suitable conditions each part of a filter is able to perform the work of any other section merely by interchanging the sections. An example of the operation of this principle is shown in Exp. 4. In this case the sixth section, which contributed very little towards the total oxidising effect of the whole filter, but was highly specialised for nitrification, was made the first section for one month. During this period microbial counts showed that there was a rapid development of yeasts, bacteria and protozoa in this section which corresponds with a parallel increase in the amount of sugar oxidised. At the end of the month as much sugar was completely oxidised to  $\text{CO}_2$  and water by the sixth section (temporarily the first) as by the section previously in the first position. Even after one month, however, the latter section was able to decompose more sugar than the original sixth section and at the same time effect a considerable nitrification. It thus appears that a filter gains in efficiency when its various parts are matured by receiving in turn the strong nutrient solution.

The sequence of changes which the sugar undergoes in its ultimate

breakdown to  $\text{CO}_2$  and  $\text{H}_2\text{O}$  is so far mainly a matter for speculation. A mixed flora exists on the filter and contains organisms which may bring about almost every known type of fermentation with the appropriate end products. In a mature aerobic filter the population co-operates in oxidising the sugar and only such organisms will exist in any quantity as can effectively make use of the sugar and of the waste products of other organisms. Intermediary products will thus tend to be oxidised as soon as they are formed. There is an alternative possibility that the usual products of fermentation are not formed to any extent under aerobic conditions of a percolating filter, or if formed are capable of rapid oxidation *in situ* by the organism which produces them. This idea gains in probability when it is remembered that typical products of bacterial fermentation are relatively stable in anaerobic cultures but break down to  $\text{CO}_2$  and water when air is admitted to the cultures. Lactic acid, for example, accumulates in anaerobic yeast fermentations but disappears when the yeast is aerated [Fürth and Lieben 1922], or when the fermented liquid is treated on a percolating filter (unpublished work). There is little doubt, however, that a considerable proportion of the sucrose is first inverted as an intermediate stage in the oxidation. There is some evidence that an acid product is also formed, but the nature of this substance is as yet unknown. It has already been stated that the drop in  $p_{\text{H}}$ —evidence on which this claim is based—is recorded as the nutrient solution passes out of the first section. The latter is thick with film, which might restrict the passage of air and so produce partially anaerobic conditions and acid fermentation in those parts of the film not exposed to air.

Turning to a consideration of the role of nitrogen in the sectional filter, it is apparent that a constant supply of available nitrogen is necessary for the development and maintenance of the biological film; the greater the amount of active film the greater the demand for nitrogen. It has been shown in large-scale filters (unpublished work) that for reasons already stated the quantity of film steadily decreases from the top of the filter to the bottom. The results of the work described in this paper also show that there is a gradient of nitrogen assimilation, or protein synthesis, corresponding to the amount of film present in different levels of the filter. When an excess of nitrogen over and above this amount is added, nitrites and nitrates are produced. These compounds appear in effluents from the middle and lower sections of the filter even in the presence of appreciable quantities of sugar. For example, pronounced nitrification has occurred over long periods in solutions containing up to 60 parts of sucrose per 100,000. Similar observations were made two years ago when, as previously stated, the filter was not divided into sections. Since the work of Winogradsky [1891], it has been held that small concentrations of organic substances inhibit or prevent nitrification. This belief has repeatedly been attacked, as, for instance, by Beijerinck [1914]. There is, in fact, much conflicting evidence in support of either view. Until recently no satisfactory explanation of these contradictory facts could be

given. Cutler [1930], however, has reported that a number of organisms capable of producing nitrite from ammonia have been isolated from Rothamsted soil and percolating filters similar to the one described in this paper. Runov [1926, 1928] and Mischustin [1926, 1928], described nitrifying bacteria which multiply in media containing organic matter. The discovery of other organisms which transform nitrites into nitrates in the presence of organic matter therefore appears to await the solution of a suitable technique for their growth and isolation. The experiments described in this paper suggest the existence of such organisms.

#### SUMMARY.

1. The experiments described have been carried out with a percolating filter. This consisted of six independent sections, each 6 inches diameter and 9 inches deep, and filled with 1/8 inch to 1/4 inch clinker.

2. When a mixture of 0.033 % sucrose, 0.033 % acetic acid and 0.033 % lactic acid is fed to the filter described in (1) at a suitable rate of flow, it is readily oxidised by a mixed microbial population. A biological film is built up and the organic matter is oxidised even if the C : N ratio of the nutrient solution is 80 to 1.

3. If a 0.1 % solution of sucrose is allowed to trickle through a mature portion of the sectional percolating filter, some formation of acid and invert sugar takes place. For acid to be produced the  $p_H$  of the nutrient solution must not be less than 6.7 to 6.8. The maximum  $p_H$  drop recorded has been from an initial  $p_H$  of 7.0 to one of 6.6. The nature of the acid product is as yet unknown. It is suggested that the acid arises either as a normal intermediate product of sucrose oxidation which is destroyed almost instantaneously, or that its presence is due to the growth of a bulky film within which an acid, anaerobic fermentation occurs.

4. When the sectional filter was working well within its capacity, *i.e.* more than able to oxidise the organic matter with which it was supplied, it removed sucrose from a 0.1 % solution in approximately the following manner: Section No. 1, 40 %; No. 2, 25 %; No. 3, 12 %; No. 4, 10 %; No. 5, 7 %; No. 6, 6 %; total 100 %.

5. Each section of the percolating filter may be replaced by any other without the filter losing in efficiency. In fact the total working capacity of the filter could probably be increased by making each section the first in turn for a few weeks.

6. Oxidation of ammonia proceeds best in the lower sections where the concentration of sugar is least. It is not inhibited by concentrations of sugar up to 0.05 to 0.06 %. Therefore nitrification and carbohydrate oxidation take place simultaneously in several of the sections. Ample evidence exists in support of the view that bacteria which nitrify ammonia may be active in presence of organic matter.

7. Both nitrites and nitrates are produced by micro-organisms in the presence of 3 to 4 parts per 100,000 of nitrogen as ammonia.

8. Each section, irrespective of its previous function, rapidly shows nitrifying activity when placed in a suitable part of the filter.

The unpublished results referred to in the Introduction were obtained by Messrs E. H. Richards, R. B. Dawson, S. W. Johnson, J. T. Martin and the author. Mr E. H. Richards, Head of the Fermentation Department, has been personally responsible for the direction of the work described in this paper and his keen interest and stimulating criticisms have been highly valued.

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# CLIV. THE BIOLOGICAL FILTRATION OF DILUTE SUCROSE SOLUTIONS.

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## 1. INTRODUCTION.

IN a recent paper on this subject Jenkins [1931] has shown that in 0.1 % solution either pure sucrose, or mixtures of sucrose and lactic and acetic acids, are readily oxidised on a percolating filter containing  $\frac{1}{8}$  to  $\frac{1}{4}$  inch clinker to a depth of 54 inches when fed at a suitable rate of flow. The suitability of soluble carbohydrates for the nutrition of micro-organisms needs no emphasis and the more or less complete exhaustion of such solutions is only to be expected provided a sufficient number of the right type of organisms is present under suitable conditions for a sufficient period of time.

The fundamental factors in biological filtration are obviously

- (1) the microbial population;
- (2) the conditions under which this population exists as determined by
  - (a) the size of the particles constituting the medium,
  - (b) the rate of flow of the liquid;
- (3) the concentration of the nutrient solution.

The object of these investigations was the more precise determination of the above factors and their application to the treatment of some 250,000 gals. per day of liquor containing 0.2 % of sugar in solution.

The rate of flow used by Jenkins was 9 cc. per minute, equivalent to 86 gals. per cubic yard of filter bed per day. The treatment of 500,000 gals. of a 0.1 % sugar solution at this rate of flow would require a filter bed five-sixths of an acre in area with a depth of 54 inches. It is obviously of some importance to know if these dimensions are the minimum required for the effective solution of the problem.

The complete oxidation of sugar to  $\text{CO}_2$  and water requires 1.12 times its weight of oxygen, so that a 0.2 % sugar solution, *i.e.* 200 parts of sugar per 100,000, will require 224 parts of oxygen per 100,000 of water. Since the solubility of oxygen in water is 1 part by weight per 100,000, the solution will require constant aeration during its passage through the filter, and the rate at which this occurs will be a very important controlling factor in the efficiency of the filter. The rate of aeration is determined by the size of particle and the rate of flow of the liquid.

The semi-commercial scale experiments conducted at Colwick, and referred to by Jenkins, showed that the use of fine clinker of  $\frac{1}{8}$  to  $\frac{1}{4}$  inch was liable to lead to the choking of the filter bed and insufficient aeration, resulting in a very much reduced purification.

Investigations of the inter-relation between concentration of nutrients, the size of particles and rate of flow and their bearing on the rate of oxidation are described in the present paper together with an account of the nitrogen and phosphate requirements of the biological film.

## 2. THE FIVE-DAY TEST.

This test measures the polluting value of an effluent by means of the biological oxidation of the organic matter. It therefore achieves the object of the filtration process. For this reason a study of the test will help towards a better understanding of biological filtration.

A sample of the water to be tested is diluted with water saturated with air at atmospheric pressure and  $18^{\circ}$ . By choosing a suitable degree of dilution the water will supply sufficient oxygen for the oxidation of the organic matter, provided of course that the right organisms are present. Determinations are made by Winkler's method of the dissolved oxygen in the water at the beginning and after 5 days' incubation at  $18^{\circ}$ , the difference being the amount of oxygen used in the oxidation of the organic matter.

With the object of testing the degree of correlation between sugar content and oxygen absorbed, 5-day tests were carried out on 0.2, 0.15, 0.10 and 0.05 % sugar solutions. The results are given in Table I, dilutions of 1 in 200 being used in each case.

Table I.

Concentration of sucrose solution %	Dissolved oxygen available in parts per 100,000	Dissolved oxygen required in parts per 100,000 (Calculated)	Dissolved oxygen absorbed in parts per 100,000 (Actual)	Sucrose accounted for %
0.20	180	220	131	60
0.15	180	165	102	62
0.10	180	110	62	56
0.05	180	55	35	63

In every case the amount of oxygen absorbed is little more than half the amount required for complete oxidation of the sugar. This discrepancy may be accounted for by (a) sugar undecomposed, (b) sugar metabolised but not oxidised. To what extent each of these two factors is responsible for the low oxygen absorption would be very difficult to determine. Assuming the unabsorbed oxygen to be due entirely to undecomposed sugar the maximum amount of sugar would be 0.0004 %. Actually most of it will exist as decomposition products of sugar and as cell tissue of the organisms effecting the decomposition. A more extended incubation period is obviously necessary for more complete oxidation. At the extreme dilutions of sugar used in this test the generation time of the organisms must be very considerable, probably 12 hours

or more (see p. 1432). Another series of tests was carried out in duplicate with the same dilution using a 0.15 % sugar solution. Ten bottles were incubated in each case and the temperature of incubation raised from 18° to 25° during the first 5 days. Determinations of oxygen absorbed were made at intervals during 20 days. The results are given in Table II.

Table II.

	Present at start	Dissolved oxygen in parts per 100,000 after								
		1	2	3	4	5	6	7	10	20 days
I	142	92	55	35	27	24	24	22	19	2
II	142	94	56	35	26	24	24	23	19	2

These results show that the absorption of oxygen is most rapid during the first 3 days and that 70 % of the sugar is oxidised after 4 days' incubation. This result is considerably higher than that obtained in the previous experiment.

Cook and Stephenson [1928] using pure cultures of *B. coli* measured the oxygen absorption in a Barcroft apparatus and found that for glucose only two-thirds of the theoretical amount was absorbed by the organism. They offer no explanation to account for the incomplete oxidation of the glucose and do not consider that bacterial growth may be responsible. In the 5-day test, however, there is an increase in bacterial numbers during the first 2 days and the writer is of opinion that bacterial growth and synthesis is responsible for the incomplete oxygen uptake. The differential rates of oxygen uptake as well as the different amounts recorded by Cook and Stephenson [1928] for various carbohydrates seem to point to a difference in value of these carbohydrates in metabolic synthesis (see p. 1431). This subject is being investigated in greater detail.

The determination of the possible variation due to the inoculation was made by preparing a series of tests using tap water only for dilutions, and another series with the addition before dilution of 1 % of a laboratory filter effluent to the sugar solution to be tested. The results are given in Table III.

Table III.

No. of sample	Oxygen absorbed per 100,000	Standard deviation
1	91	Mean = $92 \pm 7.4$
2	87	
3	89	
4	108	
5	87	
6	89	
7	112	Mean = $110 \pm 3.1$
8	109	
9	115	
10	107	
11	113	
12	106	

These results show that leaving the dilution water to a chance inoculation causes a decreased oxygen absorption and a greater variation in the amount than using a definite inoculation from a filter bed.

The standard deviation of the mean using a definite inoculation is 3.1 which means that a single determination may vary as much as 9.0 % from the mean value.

As an accurate method of determining the amount of organic matter present in a liquid this test is obviously not very reliable. A controlled inoculation is not practicable since there is no known method of producing cultures of bacteria in a uniform state of efficiency.

The possibilities of a chemical test answering the purpose are not encouraging. Recent work at Rothamsted has shown the 4-hour permanganate test to be unsuitable for estimating the oxygen requirement of organic acids [Water Pollution R.B. Report, 1929]. The only reliable method is that of combustion, and this could not be considered as a true measure of the polluting effect, since there are organic substances which are comparatively inert biologically. There appears therefore to be no alternative to this incubation test, and, provided too much reliance is not placed on single determinations, it may be considered to answer the purpose for which it was designed. In the succeeding experiments it has been used as a measure of the amount of purification effected by biological oxidation on percolating filters. In this case the calculations are made from determinations obtained by using identical inoculations.

### 3. THE BIOCHEMICAL OXIDATION OF SUCROSE.

A very extensive literature exists on this subject which for the purposes of this paper may be summarised as follows.

After hydrolysis of the sucrose the hexose sugars give rise to methylglyoxal ( $\text{CH}_3\text{.CO.CHO}$ ) which yields lactic acid ( $\text{CH}_3\text{.CHOH.COOH}$ ) and then by oxidation pyruvic acid ( $\text{CH}_3\text{.CO.COOH}$ ). Pyruvic acid on hydrolysis yields acetic and formic acids. Formic acid is readily decarboxylated yielding  $\text{CO}_2$  and  $\text{H}_2$ . Alternatively pyruvic acid may be decarboxylated yielding acetaldehyde which on reduction yields alcohol and on oxidation acetic acid. Acetaldehyde may also form the starting-point of several organic syntheses by polymerisation and condensation forming higher fatty acids and alcohols.

In biological filtration any or all of these compounds may be produced according to the bacterial population and conditions of aeration of the filter bed. In any case the fundamental processes in all the different types of fermentation are the same, and the object of filtration, *i.e.* the complete oxidation of the carbohydrates to  $\text{CO}_2$  and water, is best secured by reducing the synthetic reactions to a minimum. In accordance with Hopkins's dictum the production and accumulation of any particular compound in the filter bed, other than the final products of oxidation, is a measure of its uselessness to the organisms producing it [Stephenson, 1930]. From the point of view of

pollution the intermediate products, lactic and acetic acids, theoretically have the same oxygen absorption value as sugar, so that their production does not result in any reduction in the polluting effect of the liquid. Their decomposition requires the presence of hydrogen acceptors which may be provided either by free oxygen or oxygen combined as nitrate [Quastel, Stephenson and Whetham, 1925]. Since the solubility of oxygen in water is very slight, it may become the limiting factor determining the rate of oxidation. It has long been known that denitrification is part of the respiratory process of bacteria under conditions of restricted aeration. The presence of oxidised nitrogen either as nitrites or nitrates in a filter bed is generally considered as indicating its efficient working, but the possibility of their playing a part in the actual oxidation of carbohydrates does not appear to have been previously recognised.

#### 4. EXPERIMENTS WITH SECTIONAL FILTERS.

With the object of obtaining more information as to the working of a biological filter, a special filter was designed by the writer and manufactured in earthenware by Messrs Doulton of Lambeth. The principle of this filter was adopted by Jenkins and described in a recent paper [1931]. The original apparatus consists of pipe sections each 1 foot in length and 4 inches in diameter fitted at one end with a perforated plate containing 5 holes  $\frac{1}{2}$  inch in diameter. Each section was set on a separate earthenware saucer provided with a single drainage hole 1 inch in diameter. The object of the saucer was to collect the effluent from each section and deliver it to the centre of the next section below. In this way the possibility of the liquid passing down the inner surface of the pipes was obviated. A certain amount of spreading of the effluent over the surface of each section was effected by the presence of a ring of drip nipples round the lower edge of the drainage hole of each saucer.

The capacity of each section, assuming a 9 inch working depth, was such that when supplied with 1 litre of liquid per 24 hours it was equivalent to a filter bed receiving 100 gallons per cubic yard per day (g.y.d.). Five such sections placed in a column can be conveniently supplied with 5 litres of liquid per day from a 6-litre aspirator at this unit rate of feed. The possible criticism that such a filter would be subject to abnormal conditions on account of the supposed increased aeration due to the air gaps between each section is not valid. The contraction of the film in passing from one section to another would result in less aeration than if the filter medium were continuous. It should be remembered, however, that the choking of any one section not only affects that particular section, but in practice would also put out of action the whole column.

In filtration experiments the rate of flow of the liquid through the bed is obviously of great importance and precautions must be taken to ensure that the rate of flow is actually what was intended. In these experiments the aspirators were fitted with a Mariotte's constant head device which released

the liquid under a very low uniform hydrostatic pressure. The use of a "T" piece outlet obviated the possibility of gravitational pull by the dropping liquid and at the same time facilitated cleaning operations. The apparatus was kept free from both organic and mineral deposit by rinsing with hydrochloric acid each day. Before sampling, checks on the rate of flow were made by measuring the volume of effluent collected in 30 minutes.

*A. The size and nature of the filter medium.*

In this experiment two columns each of six sections were used. One column was filled with fine gravel of  $\frac{1}{4}$  to  $\frac{3}{8}$  inch and the other with clinker of  $\frac{3}{8}$  to  $\frac{5}{8}$  inch. Each column was inoculated with 100 cc. of a suspension of film obtained from a large scale experimental filter being operated at a Sugar Beet Factory. Both laboratory filters were fed with a solution of the following composition at the basic rate of 100 g.y.d.

			%
Sucrose	...	...	0.2
NaCl	...	...	0.03
K <sub>2</sub> HPO <sub>4</sub>	...	...	0.03
KH <sub>2</sub> PO <sub>4</sub>	...	...	0.02
MgSO <sub>4</sub>	...	...	0.01
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	...	...	0.03
CaCO <sub>3</sub>	...	...	0.03 (supplied by the tap water).

The  $p_H$  was 6.8–7.0.

This experiment was run continuously for a period of 60 days. Samples of the effluents of each section were examined daily for  $p_H$  and mineral nitrogen and weekly for degree of purification by means of the 5-day test.

(a)  $p_H$  values. These were determined by means of the B.D.H. capillators, with bromocresol purple and bromothymol blue. The results were plotted as curves with the numbers of the sections as ordinates and the  $p_H$  values as abscissae. During the first week little or no change in the reaction of the liquid occurred in its passage down the filters so that the graph is almost a straight line. During the second week the  $p_H$  of the effluents from the second section became more acid (see Figs. 1 and 2).

By the end of the second week acidity developed in the effluent from the first section and continued to increase during the third and fourth weeks, but to a greater extent in the effluent from the gravel filter than in that from the clinker. This difference in acidity between the two filters continued throughout the remainder of the experiment. The  $p_H$  values of sections 1 and 2 of the gravel filter averaged 5.5 whilst in the corresponding clinker sections the average was 6.0. The effluents were always less acid from section 3 and generally reached neutrality in section 4. In sections 5 and 6 they became slightly more alkaline ( $p_H$  7.0–7.1) than the original solution supplied from the aspirator. This development of alkalinity in the lower sections diminished after the 30th day with the increase in nitrification, more especially in the case of the



clinker filter in which the effluent gradually became more acid ( $p_H$  6.5) after leaving section 4 (see Fig. 2).

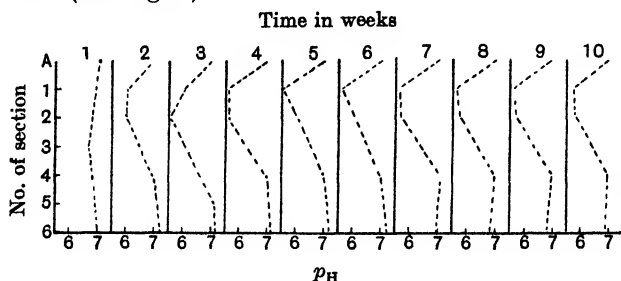


Fig. 1.  $p_H$  of gravel filter.

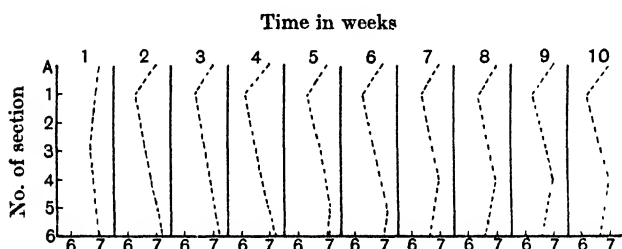


Fig. 2.  $p_H$  of clinker filter.

The greater acidity developed in the gravel filter may be due to deficient aeration, unless some basic material in the clinker was exerting a neutralising effect.

An attempt was made to diminish the acidity of the gravel filter by placing glass dishes filled with broken chalk beneath the drainage holes of sections 1 and 2. Although the chalk was at first rapidly corroded by the acid liquor there was no significant change in the reaction of the effluents. After two days the chalk was covered with a gelatinous film which prevented any further corrosion. On testing the liquid in the dishes containing the chalk it was found that the  $p_H$  was even less than that of the liquid dropping on to it from the section above. The same lowering of the  $p_H$  also occurred on collecting the effluent in glass dishes without any chalk. In 2 hours the  $p_H$  dropped from 5.5 to 5.0 and in 20 hours 4.2 was recorded. The liquid at the surface gave a  $p_H$  value of 5.8. This higher  $p_H$  of the surface layer could only be accounted for by greater aeration.

In order to confirm the improbability of any buffering effect of the clinker medium the basicity of the media was determined by titration with both hydrochloric acid and acetic acid.

With both mineral and organic acids the gravel contains more basic material than the clinker. The difference between the two is greater in the case of the organic acid on account of the occurrence of sulphides in the clinker which are not decomposed by acetic acid.

Time of shaking (hours)	Gravel cc. of <i>N</i> /10 HCl acid neutralised	Clinker cc. of <i>N</i> /5 acetic acid neutralised
24	7.3	5.8
48	9.0	8.8
72	9.4	9.15
(Some H <sub>2</sub> S was evolved by the clinker.)		
	cc. of <i>N</i> /5 acetic acid neutralised	
24	3.7	0.5
48	8.0	0.6
72	8.6	0.7

It must therefore be concluded that the difference in reaction between the two filters is due entirely to aeration and not to chemical composition. Jenkins [1931] states that "for acid to be produced the  $p_H$  of the nutrient solution must not be less than 6.7 to 6.8." It is difficult to reconcile this statement with the above observations or with any known theory of fermentation.

The cause of this difference in aeration must be attributed to the size of the particles of gravel. Although the total volume of interstitial space is independent of the size of particles, the permeability to water is proportional to the square of their diameter [Green and Ampt, 1912]. With small particles the interstitial space is also diminished to a greater extent by the film of liquid and bacterial slime. After running for 45 days the first section of the gravel filter was completely blocked with film and the liquid ran over the side of the section. This filter was therefore considered to be out of action since the lower portions only worked in virtue of their being sectional. The clinker filter continued working in a satisfactory manner for another month.

The gravel filter was therefore dismantled and the amount of film present in each section was determined in the following way.

(b) *Determination of weight of film in gravel filter.* The gravel from each section was shaken thoroughly with successive lots of 400 cc. of water and the total volume of suspended film made up to 2 litres. This was allowed to settle and 1 litre of clear water was decanted. The remaining litre of film suspension was then thoroughly agitated and 60 cc. of it were evaporated to dryness, dried at 100° and weighed again. It was then ignited and the ash determined.

The results are as follows.

Table IV.

Section	Total weight of film g.	Weight of ash g.	Weight of volatile matter g.	Ash %
1	14.6	4.8	9.8	33
2	14.0	6.2	7.8	44
3	11.4	6.2	5.2	54
4	9.4	5.2	4.2	55
5	12.6	7.2	5.4	57
6	5.2	3.4	1.8	65

These results show

(1) that the greatest amount of film occurs in the first section and that

the amount generally diminishes with depth; the irregularity of the fifth section is probably due to collection of film discharged from higher sections;

(2) that the ash generally increases in percentage amount with depth and constitutes the bulk of the film in the lower sections.

(c) *Reconstructed gravel filter.* This filter was then reconstructed with coarse gravel as follows:

Section 1	}	$\frac{1}{2}$ to $\frac{3}{4}$ inch	Coarse.
„ 2			
„ 3	}	$\frac{3}{8}$ to $\frac{1}{2}$ inch	Intermediate.
„ 4		$\frac{1}{4}$ to $\frac{3}{8}$ inch	Fine as before.
„ 5			
„ 6	}	$\frac{1}{4}$ to $\frac{3}{8}$ inch	Fine as before.
„ 6			

It was then inoculated with the wash water from the previous medium.

As in the previous experiment acidity first developed in section 3 at the end of the first week, after which it occurred only in the first ( $p_H$  5.6) and second sections ( $p_H$  6.2). After the second week the  $p_H$  of the first section varied between 6.0 and 6.5 whilst that of the second section rarely fell below 6.5. The  $p_H$  curves obtained with this graded gravel were almost identical with those obtained with the clinker filter, thus confirming the conclusion that for the above experiment the size of the particles and not their chemical composition or texture determines the development and efficiency of the film.

Both filters continued to operate in a very satisfactory manner for 20 weeks when both were choked in the lower sections by copious discharge of film from the sections above.

(d) *Purification.* The purification effected by a filter is calculated from the oxygen absorption figures in the 5-day test of the original solution and of the effluent. The difference between these two determinations expressed as a percentage of the first is the percentage purification. The results obtained during the first period of the two filters are given in Table V.

Table V.

Section	Percentage purification								week
	1st	2nd	3rd*	4th	5th	6th	7th	8th	
	Gravel filter.								
1	42	43	16	21	22	21	3.3	15	
2	58	77	27	46	40	55	23	40	
3	55	90	43	78	66	68	41	32	
4	68	92	57	86	87	82	66	77	
5	68	90	69	96	95	84	85	87	
6	72	90	76	93	96.5	84.5	89	94	
Clinker filter.									
1	27	52	42	60	22	38	34	36	
2	30	74	59	78	40	46	55	66	
3	52	76	68	90	54	65	80	93	
4	58	76	73	93	81	77	90	98	
5	72	86	81	96	90	85	90	100	
6	73	90	79	97	91	93	95	100	

\* Nitrogen omitted.

These results lead to the following conclusions.

(1) There was no difference between the filters during the first 3 weeks. Both gravel and clinker media gave 90 % purification after 2 weeks.

(2) Omission of nitrogen and consequent retardation of the growth of film lowered the efficiency immediately.

(3) Most of the purification was effected in the first three sections and very little effect was produced by the sixth section (see Fig. 4).

(4) The fine gravel filter showed a fall in efficiency in the first section after the second week, but without any appreciable effect on the lower sections.

The changes in the purification of each section are shown in Figs. 3 and 4.

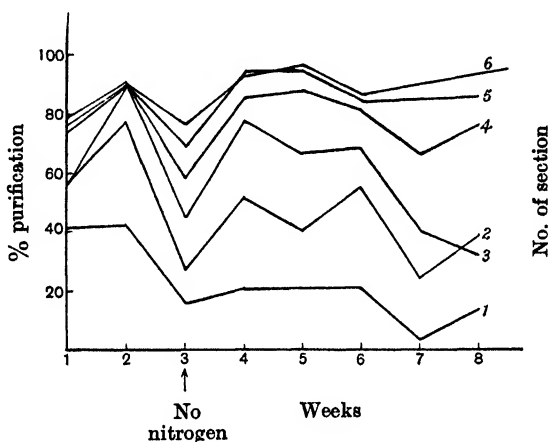


Fig. 3. Purification curves for fine gravel filter.

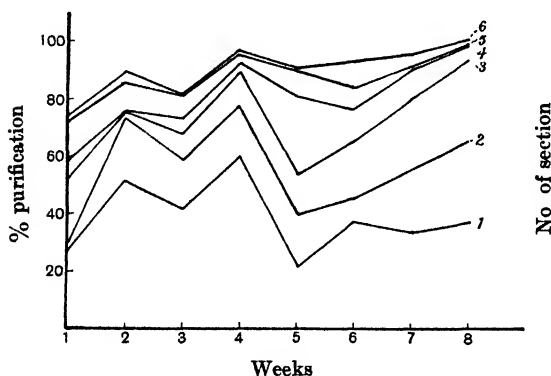


Fig. 4. Purification curves for clinker filter.

The purification results of the second period after reconstructing the gravel filter are as follows (Tables VI and VII).

These results show:

(1) that the coarse gravel ( $\frac{1}{2}$  to  $\frac{3}{4}$  inch) was much superior to fine gravel ( $\frac{1}{4}$  to  $\frac{3}{8}$  inch) and to clinker ( $\frac{3}{8}$  to  $\frac{5}{8}$  inch); the purifications in the first two

Table VI. *Coarse gravel (regraded).*

Section	Percentage purification							
	1st	2nd	4th	6th	9th	11th	13th	15th week
1	26	64	61	77	68	20*	46	69
2	45	88	74	86	85	73	69	90
3	60	90	84	91	86	85	82	92
4	75	94	93	95	89	95	92	95
5	83	94	95	95	96	95	95	95
6	85	95	94	95	96	95	97	97

\* Drainage outlet closed by pebble.

 Table VII. *Clinker.*

Section	Percentage purification					
	1st	3rd	5th	9th	12th	14th week
1	22	26	22	20	45	32
2	64	69	33	35	55	43*
3	80	85	59	40*	67	72
4	86	95	87	90	92	90
5	95	97	90	95	96	95
6	97	97	95	99	98	98

\* Choked with larvae and debris, and cleared.

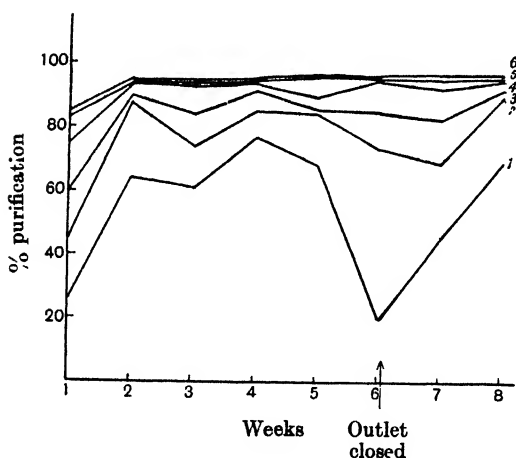


Fig. 5. Purification curves of regraded (coarse) gravel filter.

sections were much higher and showed less falling off in efficiency with age (Fig. 5);

(2) that starting the filter with the previous inoculation showed more rapid development of purification than with the original film from the large scale filter bed;

(3) that 3 feet of gravel ( $\frac{1}{2}$  to  $\frac{3}{4}$  inch) will give 90 % purification of 0.2 % sucrose solution after 2 weeks and maintain this for more than 16 weeks;

(4) that very little extra purification was obtained after the 4th section in either filter.

B. *Oxidation of lactic and acetic acids.*

The difference in acidity and purification between the coarse and fine filter media suggested a possible correlation between  $p_H$  and purification. An experiment was therefore carried out using 0.2 % solutions of lactic and acetic acids in place of sugar. The experiment ran for 14 days and was characterised by remarkably good purification figures and nitrification.

The results are given in Tables VIII and IX.

Table VIII. *Oxidation of lactic acid.*

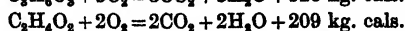
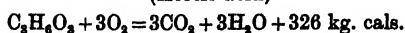
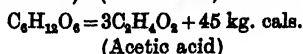
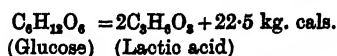
Aspirator	$p_H$	$[H'] \times 10^7$	Purification %		
			2nd day	7th day	14th day
Section 1	3.7	2000	—	—	—
" 2	4.8	158	16.6	43	58
" 3	6.5	3.2	63.0	83	83
" 4	6.9	1.1	85.0	91	99
" 5	7.0	1.0	90.0	98	100
" 6	7.1	0.8	93.0	99	100
" 6	6.9	1.1	96.0	99	100

Table IX. *Oxidation of acetic acid.*

			Purification %				
			2nd day	7th day	14th day	21st day	
			0.2 % acetic acid			0.2 % sucrose	
	$p_H$	$[H'] \times 10^7$					$p_H$
Aspirator	4.0	1000	—	—	—	—	6.9
Section 1	4.6	250	40	36	38	44	6.6
" 2	6.5	3.2	65	82	85	57	6.8
" 3	6.8	1.6	87	94	97	78	6.9
" 4	7.0	1.0	90	96	98	91	7.0
" 5	7.1	0.8	93	96	98	98	6.9
" 6	7.0	1.0	95	98	98	98	6.9

The reduction in  $[H']$  is seen to be much greater in the first section than in any of the other subsequent sections, but the amount of purification effected is about equal in the first and second sections. Obviously the reduction in acidity is effected by other processes in addition to oxidation. The results show very conclusively that the development of acidity in a filter bed is not in itself detrimental to its efficiency. If anything, it appears that organic acids are more readily oxidised than sugar, a result which is not in agreement with conclusions reached by Jenkins [1931]. It follows therefore that the low purification of the fine gravel filter is not due to any possible toxic action of the organic acids, but solely to lack of oxygen. These results are also of great interest biologically since the protozoan population of sections 1 and 2 was almost entirely extinguished, so that the more efficient filtration process was carried on by bacteria and fungi supporting a population consisting of eel-worms and insect larvae.

The more rapid oxidation of organic acids compared with that of sugar may have a possible explanation in terms of energy transformation.



In the fermentation of sugar to lactic and acetic acids the organisms may derive energy for growth and metabolism without oxidising the material, but no growth is possible on lactic and acetic acids without oxidation. When sugar is supplied to a filter bed there is doubtless a development of organisms capable only of utilising intra-molecular energy, and these organisms must necessarily die out when the filter receives only organic acid, resulting in an increase in the oxidising organisms.

### C. *The development of the film.*

The active portion of the filter medium consists of bacteria and fungi supporting a very variable population of protozoa, nematodes and insect larvae. It would be difficult to follow the possible fluctuations in the bacterial flora but they are doubtless reflected in the constantly changing fauna. In a filter bed the organisms develop in the form of colonies attached to the particles of the medium. On starting up a newly inoculated filter the liquid shows an increasing bacterial population as it flows through successive sections, until a certain maximum is reached about the third section, when clumping or colonisation begins, resulting in a much reduced dispersion of the bacteria in the lower sections. This process continues and gradually appears in the upper sections until all the effluents show a marked reduction in bacterial dispersion. The effect of this is shown in Table X by the results of direct chamber counts of bacteria in the effluents.

Table X.

Bacterial numbers in millions per cc.

Section	Bacterial numbers in millions per cc.	
	1st day	14th day
1	5.6	32-52
2	32	20-45
3	206	14-29
4	84	12-13
5	36	10-16
6	36	8-14

(These figures do not represent in any way the bacterial population of the film but only the free organisms in suspension. The numbers in the film vary from 3000 to 9000 millions per g.) As the fixed colonies proliferate they may become too heavy for the point of attachment and thus break away either as a whole or in portions. These small fragments of film are carried along with the liquid, provided the filter bed is coarse enough to allow them to pass through, and they may be seen in the effluent from all sections after the first or second week of operation. Under the microscope they appear as masses

of micrococci in the form of zoogloea with occasional yeast cells and funga hyphae. Frequently they carry with them large numbers of protozoa and eelworms. The movements of the larger eelworms and annelids may also play a part in the breaking away of the colony. The colour of the film is at first white especially in the upper sections, but after 3 weeks it changes gradually to a dark brown in the lower sections. This shedding of film is a constant process in the life of the filter and is quite distinct from the periodical sloughing which is associated with seasonal climatic changes.

The rate of growth of the film will be greatest in the first section where the concentration of food is greatest and will diminish with the depth of the section. This explains the greater purification effected in the upper sections of the filter bed. According to Penfold and Norris [1912] the time of generation of *B. typhosus* in peptone increases from 60 minutes at 0.2 % concentration to 90 minutes at 0.1 % and 180 minutes at 0.05 %. A similar result was obtained by Cutler and Crump [1923] for protozoa, the concentration of the nutrient in this case being expressed in terms of bacterial numbers. With a purification of 75 % in section 3 the concentration of nutrient in this section would correspond to 0.05 % sugar and, assuming the time of generation in mixed culture to be similar to that in pure cultures, it would be about three times that of the first section, so that the film would only grow at one-third the rate. Similarly the growth rate in the fourth, fifth and sixth sections would be reduced almost to nil. The figures given in Table IV however show much greater amount of film than would be expected from these growth rates. This difference from expectation must be attributed to the film continuously shed from the upper sections being retained by the fine gravel in the lower sections. The use of a coarse medium would allow an easier passage to this discharged film. The effect of this is seen in the reconstructed gravel filter. When the first three sections were filled with coarse gravel the discharge of film into the surface of section 4 (fine gravel) was so great that it became covered in a period of 12 weeks with a solid layer  $\frac{1}{2}$  inch thick.

#### D. *Time of contact.*

The development of the film has an effect on the filter apart from its increase in population, *viz.* in increasing the "time of contact" or the length of time the liquid takes to pass through the filter. This factor is usually determined by Clifford's method [1907] which measures the mean time required to wash out a quantity of sodium chloride from the filter at a given rate of flow.

It is obvious that the time of contact will increase with the development of the film. The film consists of about 97 % by weight of water and the total volume in one of these experimental filters may be  $1\frac{1}{2}$  litres. With a rate of flow of 5 litres per 24 hours the water of the film can be changed  $5\frac{1}{2}$  times in 24 hours which gives a time of 7.2 hours for each change of water.

The volume of film in each section is measured by the difference between



the volume of the interstitial space of the clean gravel, and that after development of the film. This determination was carried out on one of the sectional filters filled with gravel of  $\frac{1}{2}$  to  $\frac{3}{4}$  inch after running 1 month. The results are given in Table XI. The time of contact is the ratio  $\frac{\text{volume of film}}{\text{rate of flow}}$ , since if  $X$  = volume of film and  $V$  = total volume of liquid per 24 hours, then  $V/X$  = number of times the film is filled per day, and  $\frac{24}{V/X}$  = time of contact =  $X \ 24/V$ .

Table XI.

Section	Volume of film cc.	Rate of flow	Time of contact hrs.
1	450	5 litres per day	2.1
2	400		2.0
3	300		1.5
4	150		0.7
5	75		0.36
6	75		0.36

From the above table we find that out of a total of 7 hours of contact 5.6 hours are spent in the first three sections. The time of contact is greatest where nutrition and growth are greatest. By reducing the size of the gravel, thereby causing a retention of the film, the time of contact can be increased considerably but not without diminishing the aeration, and increased contact without aeration is of no value. Increase in the time of contact should be the result of growth rather than mechanical accumulation. A deficiency in time of contact only occurs when starting up a new filter, and an excess tends to occur with age. This initial deficiency can be best remedied by increasing the concentration of the nutrient solution thereby increasing the rate of growth of the film. If at the same time the rate of flow is diminished, the capacity of the filter will not be exceeded. As the film develops the concentration of the solution can be diminished and the rate of flow increased.

#### E. *Rate of flow.*

It has been shown that the development of film in a filter is greatest in the upper sections and that it is determined by the concentration of nutrients in the liquid. Increase in the rate of flow will diminish the time of contact. This will result in a lower purification gradient, which means a higher concentration of nutrient in the lower sections, and an increase in the development of film below the upper layer. Thus with a lower purification gradient a deeper filter becomes necessary.

A problem of long standing to the sewage engineer is the question whether it is better to filter a given amount of organic matter as a strong solution at a slow rate or a dilute solution at a quicker rate. Information on this point can be obtained from a consideration of the previous figures for the sectional purification. In Table VI by averaging the figures for the normal working periods, the 2nd, 4th, 6th, 9th, 13th and 15th week, we get the following percentage purification: section 1, 64 %; section 2, 82 %; section 3, 88 %.

Since the rate of flow through five sections is 100 g.y.d. the rate of flow through each section alone is five times as much or 500 g.y.d. A 0.2 % sucrose solution run at this rate undergoes a purification of 64 %, whilst the effluent of this section containing the equivalent of 0.072 % sucrose, run through the second section at the same rate, undergoes a purification of 18 % of  $0.2 = 0.036$  or 50 % and the effluent of section 2 containing the equivalent of 0.036 % sucrose undergoes a purification of only 6 % of  $0.2 = 0.012$  or 33 %.

Thus a reduction in the concentration of nutrient through a filter diminishes its efficiency, though the purity of the final effluent is increased. The effect of increasing the rate of flow with a reduction in concentration of sucrose was studied by means of two sectional filters filled with the same gravel,  $\frac{1}{2}$  to  $\frac{3}{4}$  inch. Both were inoculated with the same medium. One was run with a 0.2 % solution of sugar at 100 g.y.d. and the other with 0.1 % solution at 200 g.y.d. The experiment was run for 30 days after which the gravel was washed and the amount of film determined in each section.

The results of the purification are given in Table XII.

Table XII.

Section	Number of days							
	6		13		20		30*	
	0.2 %	0.1 %	0.2 %	0.1 %	0.2 %	0.1 %	0.2 %	0.1 %
Purification %								
3	90	59	90	72	88	74	72	50
6	96	90	99	98	99	97	97	83

\* Nitrogen omitted from both filters.

There was little difference between the final purification in both filters until nitrogen was omitted from the solution. But throughout the experiment the purification at section 3 was distinctly in favour of the more concentrated solution. The effect of dilution is not apparent with a filter 6 feet deep but with a shallow filter of only 3 feet the use of a more dilute liquid at increased rate of flow gives a lower purification.

The weights of film on the various sections are given in Table XIII.

Table XIII. *Distribution of film with different concentrations of sucrose.*

Section	0.2 % solution at 100 g.y.d.		0.1 % solution at 200 g.y.d.	
	Total film (g.)	% ash	Total film (g.)	% ash
1	16.5	26	15.8	26
2	16.0	32	14.9	33
3	10.4	37	12.5	38
4	6.0	42	7.8	44
5	4.3	44	5.6	57
6	4.1	46	4.5	59
Total	57.3		61.1	

It would seem that the amount of film is greater in the upper sections and diminishes more rapidly with the 0.2 % solution at 100 g.y.d. than with the

0.1 % solution at 200 g.y.d. With the more rapid rate of flow the amount of film is greater in the lower sections, part of this increase being due to a greater ash content.

*F. The influence of the C/N ratio on the film.*

The effect of omitting nitrogen from the sugar solution has been shown in the purification figures in Tables V and XII. Most of the previous work on biological filtration has been done with liquids of a high nitrogen content and having a C/N ratio less than 10. The pulp-press liquor from a beet sugar factory has a C/N ratio of about 20, but since most of the nitrogen exists as organic nitrogen it is not immediately available for the nutrition of the organisms of the filter. Such a liquor would thus resemble a liquid with a higher C/N ratio. The growth of the film in the filter bed has a certain definite nitrogen requirement without which its development will be retarded. An analysis of the film from the top and bottom sections of the gravel filter (see Table IV), is given in Table XIV.

Table XIV. *Nitrogen content of film.*

Section	Total nitrogen (Kjeldahl)	% organic matter	% nitrogen in organic matter
1	7.1	67.0	10.6 = 69 % protein
6	4.6	38.7	13.1 = 85 % protein

The percentage of nitrogen in the organic matter is high, especially in the film of section 6 which contains nitrifying organisms. It is equivalent to a protein content of 85 % so that the organisms of the lowest section have a very low non-protein content (15 %) similar to that of animals in a state of starvation. Probably they are living on their own tissues since there is practically no soluble organic matter in the liquid passing through this portion of the filter.

A C/N ratio of 20 is equivalent to a nitrogen content of 2.1 % of the weight of sugar. In order to increase this nitrogen content to that of the film in the upper section, *viz.* 10.6 %, 4/5 of the sugar must be oxidised to CO<sub>2</sub>. The remaining 1/5 of the sugar is then just sufficient to combine with the 2.1 % of nitrogen to form the organic matter of the film. If more than 4/5 of the sugar were oxidised there would not be sufficient to combine with all the nitrogen, and the excess nitrogen would be excreted. Thus, the nitrogen requirements of the film can be measured by adding the nitrogen as ammonium sulphate and noting its rate of absorption in the various sections of the filter.

For this purpose four filters were run on 0.2 % sugar solutions containing different amounts of ammonium sulphate, so as to give C/N ratios of 5, 10, 15 and 20 respectively. The effluents from the various sections were tested for ammonia with Nessler's reagent. A large excess of ammonia occurred in all sections of the filters with ratios of 5 and 10. In the filter with a ratio of 15 the excess ammonia was very slight in sections 5 and 6, whereas with a ratio of 20 no ammonia was found in sections 4, 5 and 6. It appears therefore that the limit of the nitrogen requirements of the filter medium lies between the

ratios of 15 and 20, which is in agreement with the oxidation of 85 % of the sugar and the conversion of 15 % into organic matter of the film.

To test the possibility of nitrogen passing through the filter as amino-acid, formaldehyde titrations were carried out on the effluent from sections 2 and 3. The results are given in the following table.

Table XV. *Formaldehyde titration values of filter effluents.*

Sections	C/N ratio			
	5	10	15	20
	cc. of N/10 NaOH required per 100 cc. of effluent			
2	4.3	1.8	1.1	0.1
3	3.9	1.7	1.0	0.1

The titration value is negligible except in the case of a C/N ratio of 5. A titration value of 4 cc. is equivalent to 0.0056 % amino-nitrogen or 1/3 of the nitrogen supplied as ammonium sulphate. It must be concluded that amino-nitrogen is not produced in excess of the requirements of the organisms except in the case of a low C/N ratio. Similar determinations on the effluents of the fine gravel filter showed an increase in the formaldehyde titration value from 2.0 to 3.7 when ponding occurred, which suggests that the formation of amino-acid in the filters was due to some disturbing factors: in the one case possibly excess of  $\text{NH}_4$  ions (ammonium sulphate), and in the other lack of aeration. The absence of ammonia from the effluents of the filter with a C/N ratio of 20 can therefore only be due to nitrogen shortage.

The percentage purification results obtained with the various C/N ratios are given in Table XVI.

Table XVI. *Percentage purification.*

Days	Section	C/N ratios			
		5	10	15	20
2	6	83	86	79	78
7	6	93	91	92	83
14	1	41	36	30	32
	2	68	60	71	58
	3	91	73	88	76
	4	94	91	89	84
	5	95	92	90	90
	6	95	92	92	92
21	1	58	59	56	53
	2	74	76	75	72
	3	95	82	85	86
	4	98	97	94	92
	5	99	98	97	96
	6	99	98	98	98
28	1	18	28	45	43
	2	42	57	51	61
	3	60	67	83	73
	4	81	71	87	94
	5	91	90	90	96
	6	97	96	96	97

It will be seen that in the early stage of the development of the film a low C/N ratio gives a better purification, but this difference does not persist beyond 14 days. On the 21st day there is practically no difference between the various filters as regards purification in any of the sections. On the 28th day the filters with the low C/N ratio show a distinct falling off in purification in the first three sections, but there is no significant difference between the final effluents.

The filters were dismantled after 30 days and the amounts of film on each section determined. The results are given in Table XVII.

Table XVII. *Effect of C/N ratio on dry matter of film.*

Section	C/N ratio							
	5		10		15		20	
	Film g.	Ash %	Film g.	Ash %	Film g.	Ash %	Film g.	Ash %
1	18.7	21	18.6	20	16.8	25	17.1	22
2	17.9	25	17.7	27	16.4	31	15.3	27
3	12.8	33	11.8	34	10.4	37	9.7	37
4	9.7	40	9.7	40	6.4	42	7.0	43
5	4.8	56	5.0	62	4.3	44	6.6	62
6	4.1	59	4.2	63	4.1	51	4.0	63
Total	72.3		71.0		58.4		63.3	

Total weight of sugar consumed per filter = 300 g.

Deducting the ash content of the film we obtain a total weight of film produced of 50.0 g. from the C/N 5 filter and 39.2 g. from the C/N 15 filter, representing 17 % and 13 % respectively of the weight of sugar consumed. This leaves 83 % and 87 % to be accounted for by the sugar oxidised and that remaining as impurity in the final effluent. These results are rather better than those obtained in the 5-day test (p. 1421), but inferior to the 90 % obtained by Cutler and Crump [1923] in the pure cultures with continuous aeration.

From these results it would appear that the better purification obtained in the early stages with a low C/N ratio (5-10) is due to a more rapid growth of film, which may later become excessive and diminish aeration with consequent reduction of efficiency.

Some control over the growth of film would appear to be desirable for the maintenance of efficiency. Of the various limiting factors to bacterial growth, two only appear capable of application in practice, *viz.* (1) dilution, (2) reduction of nitrogen (only applicable where nitrogen has been added). Direct biological control of the population has not yet been attempted, but experiments in this direction would doubtless supply useful information.

A reduction in growth rate of the organisms can be effected by diminution in the nitrogen supply to a C/N ratio of 100. This occurred when ammonium sulphate was omitted from the solution, but in such cases there was an immediate drop in the purification. It must therefore be concluded that a certain growth rate is essential for efficient filtration.

The maintenance of mature organisms with a high  $\text{CO}_2$  output does not appear a possible solution. The most hopeful line of biological control of the film would appear to be in the use of a metazoan population to effect a more rapid consumption and discharge of film (*cf.* acetic acid experiment, p. 1430).

### G. Nitrogen fixation.

Microscopic examination of the effluents of the various sections revealed the presence of organisms resembling the capsular and sarcina forms of *Azotobacter*, the nitrogen-fixing organism. Various workers on filtration have suggested the possibility of nitrogen fixation taking part in the processes of the filter bed. A sectional filter was therefore run for 14 days on 0.2 % sugar solution *plus* mineral salts but without added nitrogen. The tap water used for dilution contained 8 parts of nitrate-nitrogen per million, equivalent to a C/N ratio of 100. The gravel was washed before commencing the trial and a small inoculation of the previous film was used. To assist the establishment of a film 0.006 % nitrogen as ammonium sulphate was added to the first day's charge in the aspirator.

The purification figures and weights of film obtained are given in Tables XVIII and XIX.

Table XVIII. *Purification of nitrogen-free solution.*

Section	Percentage purification	
	After 4 days	After 14 days
3	52	58
6	65.5	76

Table XIX. *Weights of film from nitrogen-free solution.*

Section	Weight in g.		Total nitrogen %
	Total	% ash	
1	17.6	8.1	2.02
2	10.1	11.0	
3	12.1	14.5	
4	7.7	21.0	1.46
5	6.9	34.0	
6	5.9	35.0	

Total nitrogen of the film (Table XIX):

$$\left. \begin{array}{l} \text{From sections 1, 2 and 3} \quad 39.8 \times \frac{2.02}{100} = 0.8039 \\ \text{From sections 4, 5 and 6} \quad 20.5 \times \frac{1.46}{100} = 0.2993 \end{array} \right\} = 1.1032.$$

Nitrogen supplied in solution:

$$\left. \begin{array}{l} 5 \text{ litres} \times 0.00006 \text{ g. N per cc.} = 0.3 \text{ g.} \\ 13 \times 5 \text{ litres} \times 0.000008 \text{ g.} = 0.52 \text{ g.} \end{array} \right\} = 0.82$$

Gain in nitrogen = 0.2832 g.

Sugar passed through filter

$$14 \times 5 \text{ litres} \times 0.002 = 140 \text{ g.}$$

Assuming 70 % consumption of sugar by the organisms

Sugar consumed = 98 g.

Ratio of sugar consumed/nitrogen fixed =  $\frac{98}{0.2832} = 345$ .

These results show a small but definite gain in nitrogen similar in amount to that obtained by various workers on nitrogen fixation. The effluent contained no mineral nitrogen but probably contained a small amount of organic nitrogen in the form of living organisms, so that the total fixation of nitrogen was no doubt greater than the above figures show.

From the point of view of biological filtration nitrogen fixation cannot be said to be of any real importance. With a C/N ratio of 100 the amount of nitrogen fixed is only adequate to affect a purification of 76 % after 2 weeks' running with a 6 ft. filter. Assuming the same increase in purification with depth as in previous experiments it would require a filter at least 12 ft. deep to effect a purification of 98 % using a solution with a C/N ratio of 100.

#### H. Nitrification.

The production of nitrates in a filter effluent has always been a criterion of purification. In the experiments with sectional filters the effluents from each section were tested daily for nitrite by the Griess-Ilosvay reagent. In starting up a new filter with 0.2 % sugar a period of 20 days may elapse before any nitrite appears in the effluent. The effluents from the solutions of C/N ratio 5 and 10 showed the presence of nitrite several days before the effluent from a C/N ratio of 15 or 20, and the amount of nitrite was always greater from the solution of low C/N ratio.

The amount of nitrite produced diminishes from section 6 upwards to section 4, but none is found in the upper sections irrespective of the C/N ratio. The presence of nitrifying organisms in these upper sections has been shown by applying the Griess-Ilosvay test to the dilution water after incubation in the 5-day test. These sections therefore provide an example of the suppression of nitrification even with a C/N ratio of less than 5. It would appear therefore that the degree of purification has a greater influence on nitrite formation than has the C/N ratio [*cf.* Jensen, 1929]. When this figure reaches 85 to 90 %, *i.e.* equivalent to not more than 0.03 % sugar, nitrification may appear. It has been shown that bacterial growth is much more rapid in the upper sections, being 6 times more rapid in section 1 than in section 4. This growth would have a large oxygen requirement which would be met by the reduction of any available reducible substance. In these circumstances nitrous oxide could not persist. In sections 4, 5 and 6 aeration is generally more than sufficient to maintain the oxygen requirements of the organisms. For these reasons nitrite formation only persists during the final stages of purification.

The presence of oxidisable carbohydrates does not in itself necessarily inhibit nitrification. Nelson [1931] has recently shown that *Nitrosomonas* in

pure culture may continue to oxidise ammonia even in the presence of 10 % glucose.

In the case of the filters run on lactic and acetic acids the rate of purification was more rapid and strong nitrification appeared in section 3.

Certain pure strains of bacteria isolated from the filter bed at Colwick appeared to be capable of oxidising ammonium lactate to  $\text{CO}_2$  and nitrous acid simultaneously, but in the presence of restricted aeration the nitrite did not persist [Rothamsted, 1929].

In the case of the clinker filter supplied with a solution of C/N ratio 15, the amount of nitrite in the final effluent reached 3 parts per million on the 30th day, and increased to a maximum of 35 parts on the 50th day. With the increase in nitrification all trace of ammonia disappears from these sections. The maximum amount of nitrite-nitrogen obtained in this experiment is slightly more than half the total amount supplied to the filter as ammonia. This means that nearly half the nitrogen added with the sugar is retained in the filter as organic matter and is only slowly released by decomposition.

Considerable fluctuations in the amount of nitrite were recorded from time to time, but they were generally traceable to irregularities in the rate of flow or mechanical stoppages resulting in deficient aeration. In the fine gravel filter nitrification steadily declined after the 30th day as a result of the diminishing purification of this filter. After the regrading of the upper three sections nitrification reappeared in the lower sections and continued vigorously.

The production of nitrates was detected 10 days after the appearance of nitrites, but it developed more slowly, apparently requiring the stimulus of larger amounts of nitrite. Increase in the amount of nitrate occurred at the expense of the nitrite, until all trace of nitrite disappeared from sections 5 and 6 and only a small amount remained in section 4. The amounts of nitrate in the final effluent varied considerably from time to time from 1 part N to as much as 10 parts N per 100,000, which is more than the rate of supply of nitrogen to the filter. To some extent these variations were due to differences in aeration but not entirely. The fact that they were of a much higher order than occurred in the previous period of nitrite formation suggests that the variation is of a biological nature. Fluctuations in the C/N ratio of the original solution had no direct effect on the amounts of nitrate in the effluent.

To obtain further information on this point, section 6 of the filter was removed and treated as a separate filter. Solutions containing nitrogen in different forms and amounts were run through this section at the rate of 2 litres per day (Table XX). The solution of each substance was run through the filter for not less than 24 hours. Estimations of the three forms of mineral nitrogen were made by the colorimetric methods as described in the Ministry of Health Bulletin [1929], ammonia by Nessler's reagent, nitrite by Griess-Ilosvay reagent, and nitrate by the pyrogallol test.



Table XX.

All quantities expressed in parts per million of solution.

Source of nitrogen	Total N	Amount of N in effluent as			Gain or loss	
		Ammonia	Nitrite	Nitrate		
Ammonium lactate	200	40	4	120	—	-36
Nil. " only	150	100	8	44	+ 2	—
" "	—	—	0.5	40	+ 40	—
" "	—	—	1.0	24	+ 25	—
Ammonium lactate	60	40	1.0	4	—	-15
" "	60	30	0.2	32	+ 2	—
Urea	30	1	0.2	16	—	-13
" "	60	10	0.2	13	—	-37
Nil. Water only	—	—	—	12	+ 12	—
Ammonium sulphate	140	40	1	100	+ 1	—
" "	60	50	—	16	+ 6	—
Sodium nitrite	40	—	15	40	+ 15	—
" "	20	—	0.2	40	+ 20	—
" "	60	—	18	120	+ 78	—
Nil. Water only	—	—	—	20	+ 20	—
Sodium nitrite	60	—	8	80	+ 28	—
" "	100	—	15	120	+ 35	—
" "	100	—	0.2	200	+ 100	—
" "	100	—	10	160	+ 70	—
" "	100	—	0.2	160	+ 60	—
" "	180	—	48	320	+ 188	—
Nil. Water only	—	—	—	32	+ 32	—
" "	—	—	—	24	+ 24	—
Sodium nitrite	100	—	16	160	+ 76	—

From these results it appears that the only nitrogenous substance capable of stimulating nitrate formation is sodium nitrite, which also gives rise to more nitrate-nitrogen than is supplied as nitrite. The increased nitrogen excreted in presence of nitrite is more than can be accounted for by the normal oxidation of the nitrogen in the film, since water alone extracts much smaller amounts of nitrate-nitrogen. It is suggested that the toxicity of the nitrite exerts some selective action on the mixed bacterial flora of the filter, resulting in increased breakdown and oxidation of the film. The symbiosis between these ammonifying and nitrifying bacteria is the subject of detailed bacteriological investigation.

#### J. Phosphate metabolism of the film.

In the preceding experiments the mineral salts added to the sugar solution included 0.05 % potassium phosphate (p. 1424). The amounts of available phosphoric acid in each effluent of the sections was estimated by the colorimetric method of Fiske and Subbarrow [1925].

The results are plotted in curve A, Fig. 6, the amounts of phosphoric acid found in the various effluents being expressed as percentages of that supplied in the sugar solution.

As would be expected the absorption of phosphate is confined to the first three sections or the region of growth of the filters. In the 6th section there is a slight increase in the amount of soluble phosphate in the effluent probably due to the oxidation of organic phosphorus and the nitrification process.

These changes in phosphate content of the effluents may be correlated with changes in the  $p_H$  of the sectional effluents. In Figs. 1 and 2 it has been shown that the  $p_H$  of sections 4 and 5 tends to increase above that of the original solution, whilst in section 6 it tends to become less. The absorption of phosphoric acid by the growing film in sections 1 to 3 would result in the formation of free potash which would combine with any organic acid produced by the decomposition of the sugar. Oxidation of these salts would then result in the formation of potassium carbonate and a consequent rise in  $p_H$ . In section 6 the oxidation of organic phosphorus and nitrification would result in a lower  $p_H$  in this section.

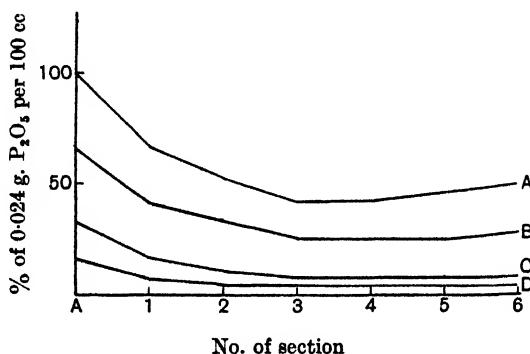


Fig. 6. Absorption curves for phosphoric acid in sectional filter.

Attempts to measure the phosphate requirements of the film by the method employed in the case of nitrogen were not successful. Reducing the amount of phosphate in the original solution even to 1/6 the amount did not result in complete absorption of the phosphate by the film (see Curves B, C and D, Fig. 6). Each reduction in phosphate supply merely diminished the phosphate gradient down the various sections. This phenomenon is probably due to the division of the phosphate between the demand for nutrition or tissue formation and the temporary requirement of the enzyme reactions involving the formation of hexosephosphate. The hydrolysis of the hexosephosphate by the mineral acid used in the colorimetric determination would account for the apparent presence of uncombined phosphate during phosphate starvation.

The effect of phosphate deficiency on the working of the filter was determined as follows.

The five sectional filters were washed and re-inoculated with equal amounts of wash water. They were then run for 3 weeks on a basic solution of 0.2 % sugar and 0.03 % ammonium sulphate to which was added potassium phosphate in different amounts as shown in Table XXI.

The highest purification figures were given by filter C receiving 0.008 %  $P_2O_5$ . Increasing the amount of phosphoric acid did not improve the filtration

Table XXI. *Percentage purification at section 3 with different amounts of phosphates in the crude liquor.*

Filter ...	A	B	C	D	E
P <sub>2</sub> O <sub>5</sub> % ...	Nil*	0.004	0.008	0.016	0.024
Days of run					
2	55	57	68	64	64
8	60	65	75	73	75
15	54	74	95	95	90
21	51	75	74	72	70

\* The tap water used in this experiment contained 0.08 part P<sub>2</sub>O<sub>5</sub> per million.

though Fig. 6 shows that it results in an increased retention of phosphoric acid by the film. This probably indicates a greater growth of film and since it is not accompanied by a corresponding increase in purification it must be assumed that aeration has become the limiting factor. This would explain the falling off in efficiency after the 15th day.

A smaller amount of phosphoric acid than 0.008 % appears to delay the development of the film, and in the almost complete absence of phosphate the film is permanently reduced in oxidising power.

#### CONCLUSIONS.

Biological filtration consists of two processes, *viz.* (1) deposition of organic matter by growth of film; and (2) oxidation through respiration. Both processes must be provided for, and since they are dependent upon one another the dominating factor in a filter bed is aeration, which is determined initially by the interstitial space and subsequently by the growth of the film. In this connection it should be realised that the minimum size of the particles is the determining factor and not the average size.

The growth of film is an essential part of the process in the filter, and any condition which delays or prevents it has an adverse effect on the purification. The most important factors influencing this growth are the concentration of food material or organic matter in the solution and its composition. Both these factors are more prominent in the initial stages of the working of the filter and their effect diminishes with age. Similarly their effect is greatest in the upper layers of the bed and diminishes with depth. An adequate supply of nitrogen and phosphorus is essential to the growth of the film and when the C/N ratio is more than 15 and the C/P<sub>2</sub>O<sub>5</sub> ratio more than 10 the development and efficiency of the film is reduced. The lower portion of the filter bed then becomes dependent on nitrogen fixation and the discharge of organic nitrogen and phosphorus from the upper layer. In the early stages both these sources of nitrogen are inadequate and consequently delayed purification results. This effect of a high C/N ratio is probably one of the chief difficulties in the treatment of sugar beet factory effluents.

Physiologically a satisfactory filter bed is characterised by the absence of putrefaction (*i.e.* the production and accumulation of readily decomposed

organic compounds). (See Table XV.) The energy of decomposition is consumed in growth so that the occurrence of dead organisms is only a momentary phase in the metabolism of the film. Considering the enormous protozoan population, the occurrence of dead organisms is very rare. Under anaerobic conditions however decomposition products may accumulate and result in a change in the  $p_H$  of the medium and a higher formaldehyde titration value.

The clumping and colonisation of the organisms in the bed of the filter is also a very characteristic feature. It results in a much lower bacterial population in dispersion through the solutions than would normally occur in a liquid culture. This clumping or agglutinating process appears to be connected with the degree of aeration. The effluents from the fine gravel filter were generally opalescent and passed through a filter paper with difficulty, whilst those from the coarse filters were clear and filtered through paper readily. The degree of opalescence diminishes with the depth of the filters.

Nitrification is an essential feature of an efficient filter bed since oxidised nitrogen provides the most effective hydrogen acceptor in biological oxidation.

Nitrite formation occurs when the concentration of sucrose and its derivatives in solution falls below 0.03 %. The absence of nitrification in the presence of a higher concentration of soluble organic matter does not imply the absence of the nitrifying organisms but rather that the potential rate of decomposition of the nitrite is more rapid than its production. The production of nitrite ultimately gives rise to the production of nitrate, and this process increases until nitrite formation is hardly detectable.

#### SUMMARY.

1. There are two limitations to the 5-day oxygen absorption test, *viz.* (a) it is not possible to estimate carbohydrate in terms of oxygen absorption by a biological process owing to organic synthesis being an essential part of the process; (b) the nature of the inoculum affects the relative amounts of oxidation and synthesis.

Recognising the above limitations, the 5-day test is the most satisfactory measure of pollution available.

2. The production of organic acids is a stage in the decomposition of sugar, but their rapid destruction is a property of an efficient filter bed.

3. The organic acids lactic and acetic are more rapidly oxidised than sucrose.

4. The formation of oxidised nitrogen in the presence of organic matter promotes the dehydrogenation (oxidation) of lactic acid and probably of other compounds.

5. The growth of film is an essential part of the purification process and the film itself accounts for 15 % of the sucrose supplied.

6. Aeration is the dominant factor in biological filtration and is determined by the minimum size of the material which provides the interstitial

space for the growth of the film and also for its discharge. A fine medium should not be used to secure time of contact.

7. If grading of the medium is desired, the smaller particles should be placed at the bottom where the smallest amount of film will develop, but to ensure free passage of discharged film nothing passing through  $\frac{1}{2}$  inch mesh should be used.

8. The rate of flow is the best means of controlling the time of contact and purification. Within limits a slow rate of flow with high concentration will increase the time of contact more rapidly than the reverse conditions. As the time of contact increases, the rate of flow may be increased without reduction in purification.

9. The growth and efficiency of film is dependent upon definite nitrogen and phosphorus requirements, *viz.* a C/N ratio of 15 and C/P<sub>2</sub>O<sub>5</sub> ratio of 10.

10. Nitrogen fixation occurs in the filter bed but results in the growth of film of low N content and low purification.

11. A low C/N ratio and a high concentration of organic matter promote the rapid development of the film and attainment of high purification. They also lead to excessive growth of the film and where possible should be used only in the first month of the working of the filter.

12. With a C/N ratio of 15 or less a filter bed 5 ft. deep will give complete purification of 0.2 % sugar solution at a rate of flow of 100 g.y.d. using coarse gravel or clinker. With a C/N ratio of 50 to 100 a much deeper filter would be required, and better results could more easily be secured by the addition of nitrogen than by deepening the filter.

13. Both stages of nitrification occur in the filter bed and are subject to considerable fluctuations not correlated with the supply of nitrogen in solution.

This subject is also being investigated from the biological standpoint by Mr D. Ward Cutler, Head of the Department, Miss L. M. Crump, and Miss A. Dixon, to whom the author wishes to express his great indebtedness for constant help in consultation and practice; also to Mr E. Hannaford Richards and the members of the Fermentation Department, and to Mr Warren of the Chemical Department, for the Kjeldahl determinations.

The investigation described in this paper was carried out at Rothamsted Experimental Station as part of the programme of the Water Pollution Research Board of the Department of Scientific and Industrial Research and is published by permission of the Department.

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# OBSERVATIONS ON SOME PARASITES OF *OSGINELLA* *FRIT* LINN.

## PART I.

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(With 14 Text-figures.)

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### 1. INTRODUCTORY REMARKS.

THE frit-fly (*Oscinella frit* Linn.) is so well known as a pest of oats and also of other cereal crops that a detailed account of its biology is unnecessary. In a few words it may be said that, under English conditions, the flies of the first generation oviposit in April and May on the leaves or stems of spring oats and on various grasses. The larvae bore into the shoots causing the death of the central leaves and growing point. Flies of the second generation oviposit during July on the ears of oats and the larvae feed upon the spikelets and young grain. Oviposition by the third generation of flies occurs during August and September or later, the eggs being laid on the leaves of winter cereals and various grasses. Winter is passed in the larval condition at the bases of the shoots which are ultimately destroyed and the over-wintering larvae give rise to the first generation of flies of the next year.

Frit-fly is especially destructive in the southern half of England, but also occurs as far north as Northumberland and has been recorded as doing damage in Scotland and Ireland. It is prevalent in most countries of continental Europe and is very widely distributed in North America.

The object of the present studies is to obtain information as to the extent to which frit-fly is destroyed by hymenopterous parasites, the species of the latter that are present and their economic significance. In carrying out these observations valuable assistance has been rendered by Dr J. Waterston of the British Museum in the identification of the parasites bred out and also by Dr H. Hedicke of Berlin with reference to the same matter.

## 2. METHODS OF INVESTIGATION.

In order to ensure an adequate supply of material for investigation, oats (variety "Supreme") were sown in 1926 and 1927 in plots set apart for the purpose on the Rothamsted farm. The plots were parts of fields already sown with oats and were consequently favourably situated with respect to becoming infested with frit-fly. Both the years 1926 and 1927 proved to be exceptional on account of the relatively slight infestation of frit-fly, and this same feature was recorded by the Ministry of Agriculture (1928) from most other parts of the country. In the Harpenden district the comparative scarcity of the insect hampered the investigation in that it greatly increased the time and labour involved in collecting material for study. The amount of material ultimately obtained was markedly less than it was anticipated would be available. During 1926 the oats were sown on a single plot on March 19th, and collections of infested plants were made at frequent intervals between June 29th and July 8th. Altogether 219 examples of frit-fly were bred and 84 parasites, the percentage of parasites being 27·7.

During 1927 three plots were used and details respecting these are given below.

*Plot I.* Date of sowing, March 15th; date of collection of pupae, June 27th; frit-flies bred, 138; parasites bred, 26; percentage of parasites, 15·8.

*Plot II.* Date of sowing, March 26th; date of collection of pupae, June 6th-7th; frit-flies bred, 128; parasites bred, 86; percentage of parasites, 40·1.

*Plot III.* Date of sowing, April 25th; date of collection of pupae, July 12th-13th; frit-flies bred, 72; parasites bred, 86; percentage of parasites, 54·4. Taken collectively 338 frit-flies and 198 parasites were bred from the three plots during 1927, the percentage of parasitism being 36·9.

On the dates indicated oat plants showing signs of infestation by frit-fly were pulled up and removed to the laboratory for examination. Each plant was then gone over and any larvae or puparia of frit-fly were removed. The puparia were carefully scrutinised and any which did not appear to be referable to the species in question were discarded. The remaining puparia were then transferred to cages containing a layer of steam-sterilised sphagnum moss. The cages were placed in an outdoor insectary, where the prevailing temperature varied only slightly from that of the open air. The cages were of an ordinary type used in rearing parasites and were little more than dark boxes with the front wall perforated for the reception of glass phials. Owing to their positive reaction to daylight, both hosts and parasites as they emerged congregated in the phials, where they were easily collected for examination.

Larvae dissected out of the stems were placed on discs of moistened filter paper and transferred to small glass receptacles and a number of them succeeded in completing their transformations. The object of keeping such larvae under observation was to ascertain whether the parasites attack their hosts while the latter are in the larval or pupal stages. From among 64 frit-fly

larvae retained in this way, 33 died, 17 produced flies and 14 produced parasites.

The above methods, although laborious, were adopted in order to make quite certain that the parasites bred were, in every case, species actually affecting *Oscinella frit*. In this connection it is noteworthy that all the flies that emerged from unparasitised pupae pertained to this species.

During 1926-7 attention was mainly confined to the parasites of the first or stem infestation of frit-fly: those of the second or grain infestation are not dealt with in the present article.

### 3. PARASITES REARED FROM *OSCINELLA FRIT*.

Six species of parasitic Hymenoptera were reared in the Harpenden district from puparia of *Oscinella frit* during the years 1926-7. Three of these species, viz. two Chalcids and a Braconid, were present only very rarely and an account of them is postponed until further material is available. The remaining three species, which are dealt with in some detail, are *Halticoptera fuscicornis* Walk. (Chalcidoidea), *Rhopstromeris eucera* Hartig (Cynipoidea) and *Loxotropa tritoma* Thoms. (Proctotrypoidea). Little is known concerning any of these three species and no detailed descriptions of them exist.

#### I. *Halticoptera fuscicornis* (Walk.).

The genus *Halticoptera* Spinola is placed in the family Miscogasteridae, a group which is doubtfully distinct from the Pteromalidae and only separable from the latter on comparatively trivial characters. *Halticoptera* occurs over the whole of Europe, Northern Africa and extends its range into both North and South America. It is closely allied to *Dicyclus* Walk. and both genera are characterised by the parapsidal furrows being incomplete and only evident anteriorly: also, in both genera the clypeus is transverse with a deep median sinus in its anterior margin. In *Halticoptera* the marginal vein is longer than the stigmal, whereas in *Dicyclus* it is usually shorter than or, at most, equal in length to the stigmal vein. The most striking difference, however, between the two genera is afforded by the maxillary palpi in the males. In *Halticoptera* the two distal joints are greatly inflated so that they form together a spheroidal vesicle, while in *Dicyclus* they are unmodified as in the females of both genera.

Published observations on the biology of *Halticoptera* are very scanty, but they indicate that it is a parasite of cyclorrhaphous Diptera and the following records are noteworthy. *H. smaragdina* (Curt.) is recorded by Curtis (1860) and by Billups (1883) from pupae of the celery-fly (*Acidia heraclei* L.) in England, but it has been doubted whether it is a primary parasite of the *Acidia* or a hyperparasite through the Braconid *Adehura apii*. Smith and Gardner (1922) recorded *H. flavicornis* Spin. as a common parasite of *Acidia heraclei* in England, a fact which lends support to the conclusion that *H. smaragdina* is a primary parasite also. *H. petiolata* Thoms. is recorded by Meyer (1923)

from *Oscinella frit* in Germany and also by Ruschka and Fulmek (1915) from Anthomyidae living in *Brassica oleracea* in Lower Austria. *H. sulius* Walk. is recorded by Schander and Meyer (1925) also from *Oscinella frit* in Germany, while *H. patellana* (Dalm.) is mentioned by Ruschka and Fulmek (1915) under the name *H. similis* Forst. as being bred from puparia of Diptera parasitising *Agrotis tritici* L in Lower Austria. Of especial interest is the species *H. daci* Silv. from Eritrea which has been introduced by Silvestri (1914) into Italy, along with other parasitic Hymenoptera, as an auxiliary biological agent in the control of the olive-fly *Dacus oleae* of which it is a

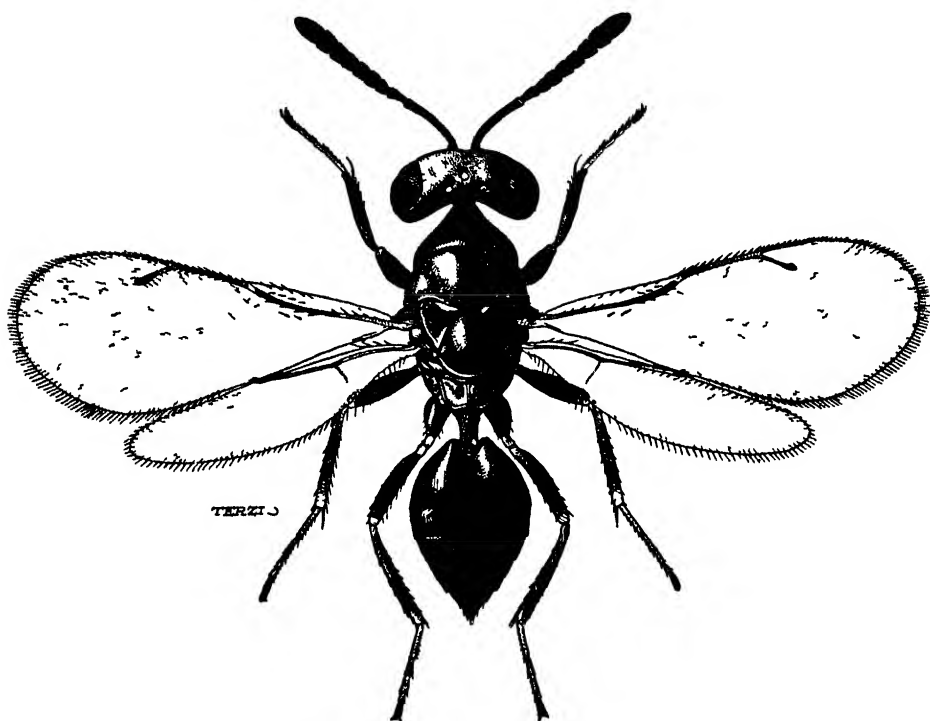


Fig. 1. *Halticoptera fuscicornis* (Walk.), female.  $\times 36$ .

parasite. The species *H. broderi* Ashm is described by Ashmead (1887) from the Cynipid *Biorhiza forticornis* (Walsh) in Canada, but it appears probable that it was parasitising a species of dipterous larva inhabiting the gall of the *Biorhiza*. Another doubtful record concerns the species *H. (Pachylarthrus) breviventris* Forst. which is stated by Dours (1874) to have been bred by Goureau from the Coleopteron *Bruchus palladicornis* Schh.; the name *breviventris* it may be added is not recognised by Dalla Torre in his Catalogue (1898).

*Halticoptera fuscicornis* was described by Walker (1833) as *Dicycylus fuscicornis*, and the first record of it parasitising *Oscinella frit* is that of Cunliffe (1921) who reared it in 1919 from puparia of *O. frit* collected in the Oxford

district. Dr Cunliffe informs me that he has again reared it from the same host in 1926; it has also been bred by Mr T. H. Taylor from frit-fly puparia in the Leeds district. From an examination of collections of frit-fly parasites reared by Messrs Cunliffe and Taylor, it is evident that *H. fuscicornis* is common in the two districts mentioned. It is also prevalent around Harpenden (Herts.) and probably distributed through the greater part of England and Wales, but this point needs enquiry. Examples reared from host puparia collected at Harpenden emerged between July 7th and September 3rd, the majority appearing during the month of August. It is evident, therefore, that the species is on the wing during the whole of summer. It attacks its host as an endoparasite in the larval stage of the latter and finally completes its transformations within the puparia.

*Description of the female* (Fig. 1).

*Coloration.* Varying from dark metallic green to bronze-black, or bronze with the abdomen dark green; hairs whitish throughout. Eyes dark chocolate; ocelli translucent brown. Antennae with scape and pedicel dark metallic green or bronze; funiculus and club very dark fuscous or brown-black. Mandibles yellow-brown, teeth darker. Legs with coxae dark metallic green to bronze-black; trochanters smoky ochreous; femora dark bronzy brown with proximal and distal extremities ochreous; tibiae and tarsi ochreous often variably suffused with fuscous, in some examples all the tibiae are strongly infuscated; fifth tarsal joint and claws brown-black. Tegulae dark brown; wings hyaline with veins pale smoky yellow. Pedicel black; abdomen smooth and shining with green or bronze reflection.

*Head.* Facial aspect broader than long (Fig. 2), as 6 : 5, sparsely clothed with short hairs; occiput broadly and rather deeply concave. Eyes moderate sized, separated by a width slightly less than frontal length of head. Ocelli disposed as a broad-based triangle; lateral ocelli separated from orbits by their own distance apart. Toruli (*T* in Fig. 2) oval, separated by about their

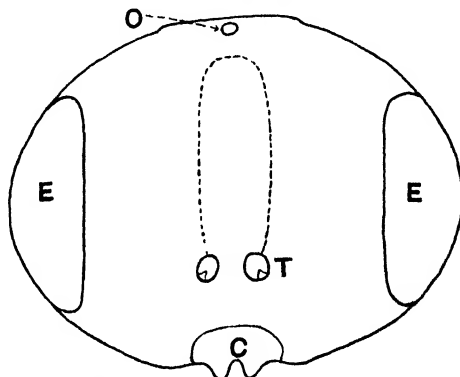


Fig. 2. *Halticoptera fuscicornis*, female: outline of frontal aspect of the head,  $\times$  circa 100.  
C, clypeus; E, compound eye; O, median ocellus; T, toruli.

own length and by nearly three times that length from a base line passing through the floor of the clypeal sinus. A rather broad, shallow median longitudinal furrow (Fig. 2) extends from behind the toruli to about three-fourths of the distance to the median ocellus. Clypeus (Figs. 2 and 3) with two projecting processes separated by a median sinus. Labrum (Fig. 3 D) sub-triangular with a median anterior process bearing a pair of greatly flattened, spatulate setae<sup>1</sup>. The whole surface of the head is sculptured with a reticulation of mostly hexagonal or pentagonal cells (Fig. 3 I).

*Antennae* (Fig. 3 A). Length 0.8 mm.; scape 0.23 mm. long  $\times$  0.03 mm. broad, related in length to pedicel as 7 : 2. Pedicel longer than any of the funicular joints which are sub-equal in length. The two ring-joints together slightly shorter than first funicular joint; club 3-jointed, related in length to last funicular joint as 7 : 2. Sensoria vary slightly in number in different examples; their approximate numbers from first funicular joint to the apex of the club are respectively 7 : 7 : 7 : 7 : 9 : 10 : 11 : 15 : 8.

*Mouth-parts.* Mandibles (Fig. 3 C) of the two sides closely alike; 0.2 mm. long  $\times$  0.12 mm. maximum breadth; each with four teeth. Maxillary palpi 4-jointed; joints related in length as 4 : 12 : 8 : 15. Labial palpi 3-jointed; joints related in length as 2 : 1 : 4. Both pairs of palpi terminated by a stiff seta, longer than third joint of maxillary palpus.

*Thorax* (Fig. 1). Tergal, pleural and sternal sclerites of pro- and mesothorax with a reticulate pattern very similar to that of the head; pubescence almost wanting. Mesonotum with parapsidial furrows incomplete posteriorly, faint when viewed dorsally but evident when seen laterally. Scutellum almost hemispherical in profile from side to side; posterior margin with a raised border. Metanotum band-like, its surface devoid of reticulation. Propodaeum (Fig. 3 H) with a median carina and a lateral carina on either side, the three carinae merging posteriorly into a transverse ridge; on each side, in front of the spiracle, is a group of rather long hairs which are continued down each side margin; posteriorly the propodaeum bears a median socket which receives the anterior end of the pedicel. Fore wings (Fig. 1) hyaline, rather more than twice as long as broad; length (four examples) 1.4 mm. to 1.8 mm., breadth 0.61 mm. to 0.82 mm. (including marginal hairs). Distal two-thirds of surface for the most part uniformly invested with sub-equal hairs; basal one-third almost hairless. Sub-marginal, marginal, post-marginal and stigmal veins related respectively in length as 14 : 7 : 7 : 4. Sub-marginal vein with 14 or 15 setae; sub-marginal cell with a proximal row of 11 or 12 setae and distally 16 irregularly arranged setae. Marginal vein with 9 to 11 macrochaetae along extreme costal edge; macrochaetae of post-marginal vein diminish in size distally and merge into ordinary marginal bristles. Stigmal vein with 4 annuli (Fig. 3 B).

Hind wing nearly four times longer than broad; average length 1.32 mm.,

<sup>1</sup> The apices of these setae vary in configuration: in some examples one or both may be slightly bifid.



breadth 0.35 mm. Marginal vein with 4 basal setae and 2 setae at the bend followed by 2 annuli; 14 to 16 marginal setae between latter and the frenulum.

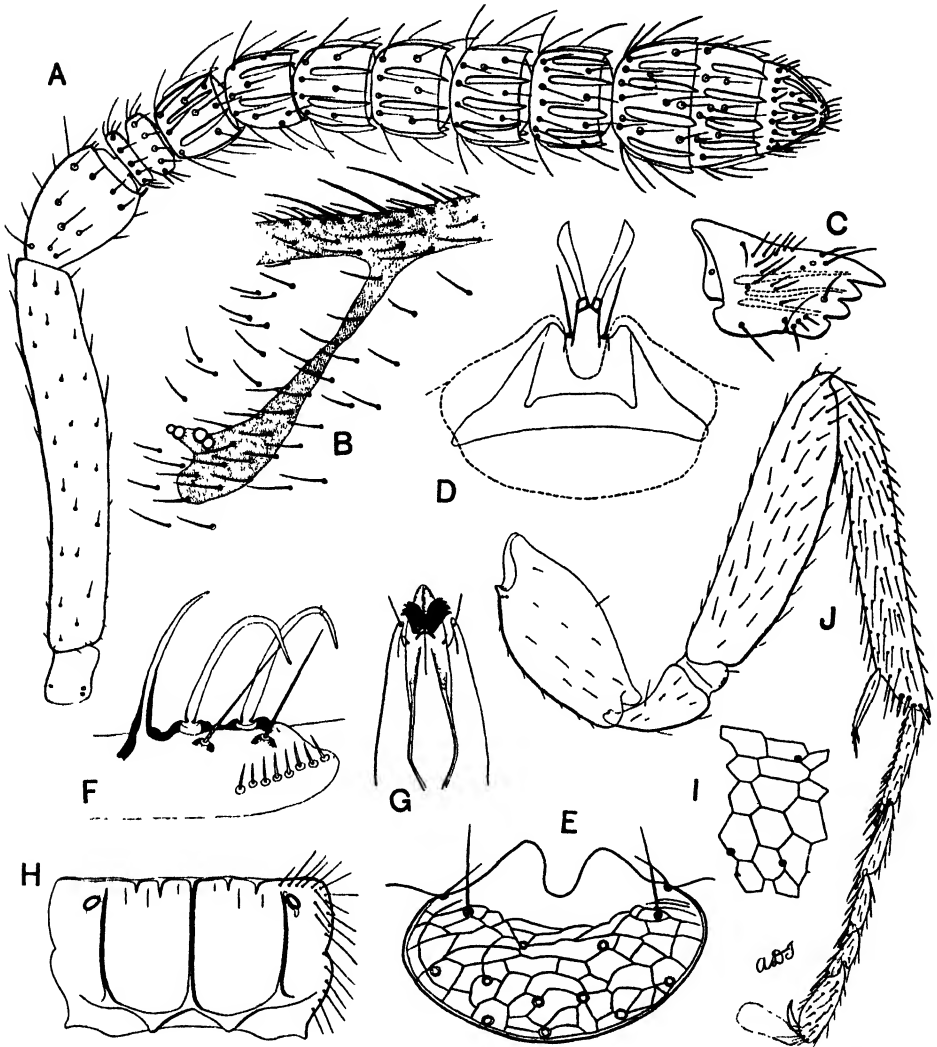


Fig. 3. *Halicoptera fuscicornis*, structural features: with the exception of G, all the parts are those of the female. A, left antenna,  $\times 232$ . B, stigmal vein and adjacent portion of costal area of left fore wing,  $\times 345$ . C, left mandible,  $\times 130$ . D, labrum,  $\times 300$  (the broken line indicates the position of the clypeus). E, clypeus,  $\times 300$ . F, frenulum of right hind wing,  $\times 900$ . G, male genitalia,  $\times 125$ . H, propodeum,  $\times 100$ . I, cuticular sculpturing of head from region about mid-way between orbit and median fossa,  $\times 300$ . J, left fore leg,  $\times 110$ .

Frenulum (Fig. 3 F) of 3 hooks; on both dorsal and ventral aspects there are 2 associated setae and, nearby, a pecten of 8 minute spines.

**Legs.** Fore legs (Fig. 3 J) with coxae moderately swollen, their length

related to the breadth as 3 : 2; second joint of trochanter small and weakly chitinised; femur broader and shorter than tibia, the latter bearing around its apex a girdle of 7 stout spines and a ventral bifid spur 0.11 mm. long; tarsal joints related respectively in length (measured along dorsal border) as 7 : 6 : 4 : 3 : 7; the first tarsal joint with a ventral pecten of about 14 spines increasing in length distally.

Middle legs with the coxae much smaller than preceding pair, their length related to the breadth as 8 : 7; second joint of trochanter firmly chitinised and merged with femur; femur related in length to tibia as 2 : 3; tibia with a slender acuminate ventral apical spur 0.11 mm. long; tarsal joints related respectively in length as 10 : 8 : 5 : 4 : 6.

Hind legs with coxae swollen and bearing 4 dorsal bristles, length of coxa related to breadth as 13 : 8; femur related in length to tibia as 2 : 3; tibia with a stout ventral apical spur 0.07 mm. long and adjacent thereto is a stout spine of about half that length, together with a group of 9 or 10 regularly arranged spines; tarsal joints related respectively in length as 10 : 7 : 5 : 4 : 7.

*Abdomen.* Petiole considerably longer than broad, its dorsal surface appearing granulate under reflected light and bearing a median longitudinal carina.

Gaster pyriform, shining, segments 1-5 smooth, 6 and 7 with the cuticle reticulated. Hairs scanty, principally on the two apical segments. First tergum nearly half total length of gaster, basally excavated to form a median fossa; second tergum slightly longer than any of remainder. Spiracles small; setigerous papillae on segment 7 projecting and surmounted by 4 hairs. Ovipositor only slightly protruding beyond apex of abdomen.

Average length 2 mm.; expanse of wings 3.25 mm.

#### *Description of the male.*

Brilliant metallic green usually with a bluish reflection, highest on the head. Eyes dark chocolate; ocelli pale translucent brown. Mandibles yellow, teeth brown; swollen apices of maxillary palpi yellow. Antennae with scape pale yellow inclining to ochreous or light fuscous distally and with the basal sub-joint smoky brown; pedicel ochreous with the base fuscous brown; funiculus ochreous, club wholly or mainly fuscous. Legs varying from amber-yellow to pale smoky ochreous especially the tibiae; coxae dark metallic green or blue-green; fifth tarsal joint and claws of first pair fuscous, of second and third pairs brown-black. Tegulae brown; wings hyaline with the veins pale yellow or inclining to a very pale fuscous tinge. Pedicel black; abdomen smooth and shining, darker than the thorax; genitalia dark fuscous.

The male is similar in size, or slightly smaller than the female but can be readily distinguished by its brilliant green colour and the pale antennae.

Apart from the genitalia (Fig. 3 G), the male differs from the female in certain small features of external structure. The most conspicuous secondary sexual characters are afforded by the maxillae which, as in other members

of the genus, differ from those of the female in the greater breadth of the stipes and in the two terminal joints of the palpi being inflated to form a conspicuous ovoid vesicle.

The reticular cuticular sculpture is more deeply impressed, especially over the head, and its cells are smaller. The first funicular joint of the antenna is shorter than any of the others and in the legs there are small differences in the relative lengths of the tarsal joints as compared with the female, viz. first legs 8 : 5 : 4 : 3 : 7, second legs 10 : 7 : 5 : 4 : 7 and third legs 9 : 7 : 5 : 4 : 8. In the fore wings there are slightly fewer setae in the sub-marginal cell, the proximal row consisting of about 10 setae and the irregularly disposed distal group consists of 8 or 9 setae.

## II. *Rhoptromeris eucera* Htg.

*Rhoptromeris* Först. is a sub-genus of *Eucoila* (*Eucoela*) Westw., an extensive genus of the family Figitidae. Species of *Rhoptromeris* are distinguishable from other representatives of *Eucoila* by the club of the antenna being 7-jointed in the female and by the fourth or fifth antennal joint in the male being usually conspicuously enlarged. Like other members of the Eucoilinae the species are very characteristic parasites of cyclorrhaphous dipterous larvae. With regard to the hosts of *Eucoila* and its sub-genera, it may be mentioned that *E.* (s.g. *Eucoila*) *keilini* Kieff. is recorded by Keilin and Pluvinel (1913) as a parasite of *Pegomyia winthemi* Meig. found living in fungi in the forest of Fontainebleau. The species *fungicola* Kieff., *agaricola* Thoms. and *schmidtii* Gir. are mentioned by Kieffer as being obtained from fungi where they presumably parasitise mycetophilid larvae. Webster and Parks record *E. hunteri* Cwfd. from *Agromyza pusilla* Meig. in Texas; Silvestri records *E. drosophilae* Kieff. from puparia of *Drosophila* in French Guinea; *E. albocincta* (Kieff.) is mentioned by Kieffer as being reared from a dipterous puparium, the species of the latter not being stated; *E. circularis* Kieff. was reared by Magretti from a gall of *Cynips kollari* in Italy and *E. siphonophorae* Ashm. is recorded by Ashmead (1887 a) from the aphid *Siphonophora cucurbitae* Middl. in the United States. The host of the last-mentioned species is doubtful and appears to require confirmation as it is possible that it may have been bred from a syrphid predator of the aphid in question. Of the sub-genus *Psichacra* Kieffer mentions the species *anomola* Kieff. as being reared by Carpentier from a puparium of *Pegomyia rumicis* R.D., while *impatiens* Say is a parasite of the horn-fly *Haematobia serrata* in North America and *pellaranoi* Brèthes attacks the Trypetid *Anastrepha fraterculus* Wied. in Argentina. Host-records of the sub-genus *Rhoptromeris* appear to be few in number. *R. eucera* Htg. was first recorded from *Oscinella frit* by Kurdjumov (1912) who described it as a new species *widhalmi*, which was subsequently shown by Hedicke (1923) to be synonymous with *R. eucera*. Kurdjumov's specimens, it may be added, were bred by Baranov in Russia. *R. eucera* (var. *tristis*) has also been recorded by Meyer from *O. frit* in Germany. In England this species

is abundant and in the Harpenden district it is the most important parasite of its host. It is also common in the Oxford and Leeds districts as is evidenced in collections of parasites reared by Messrs Cunliffe and Taylor. Dr Cunliffe informs me that he first reared it from *Oscinella frit* in 1919.

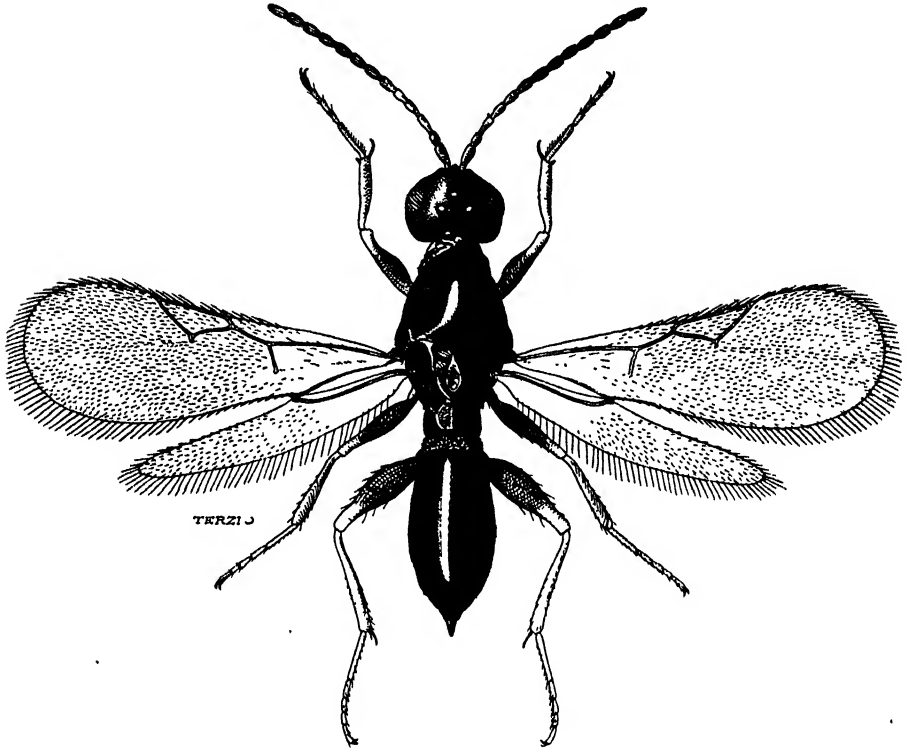


Fig. 4. *Rhoptromeris eucera* Htg., female,  $\times 40$ .

*Description of the female (Fig. 4).*

**Coloration.** Shining black, inclining to brown-black ventrally; hairs cream colour on scutellum and around the junction of thorax and abdomen, elsewhere they are mostly ashy white. Antennae with the first five joints ochreous brown or, in some examples, with the scape and pedicel dark brown; sixth joint varying from brown to dark fuscous; remaining joints very dark fuscous or black. Mandibles brown. Legs ochreous brown with the coxae dark brown; femora broadly suffused with fuscous brown or entirely of that colour; fifth joint of tarsi fuscous, in some examples the third and fourth joints are a paler shade of the same colour. Wings hyaline; veins pale yellowish brown; tegulae dark brown. Terebra ochreous brown.

**Head.** The whole surface is smooth, with very few hairs. Frontal aspect (Fig. 5) almost circular in outline, the breadth related to the length as 32 : 35. Lateral aspect ovoid, rather longer than deep (as 26 : 21). Eyes rather small, scarcely protruding and separated by a distance rather more than half the

maximum frontal width of the head. Ocelli disposed as an equilateral triangle. Toruli circular, 0.05 mm. in diameter; separated by about their own diameter and by the same distance from the orbits. Tentorial foramina conspicuous, 0.1 mm. apart. Clypeus merged with frons; its projecting frontal margin of thin chitin and provided with setae as shown in Fig. 5. Labrum (Fig. 6 L) minute, 0.02 mm. broad, membranous and clothed with very fine hairs.

*Antennae* (Fig. 6 A). Length 1.1 mm., the joints related in length as 8:5:7:6:4:4:7:7:7:7:7:8. Sensoria present from the seventh joint upwards.

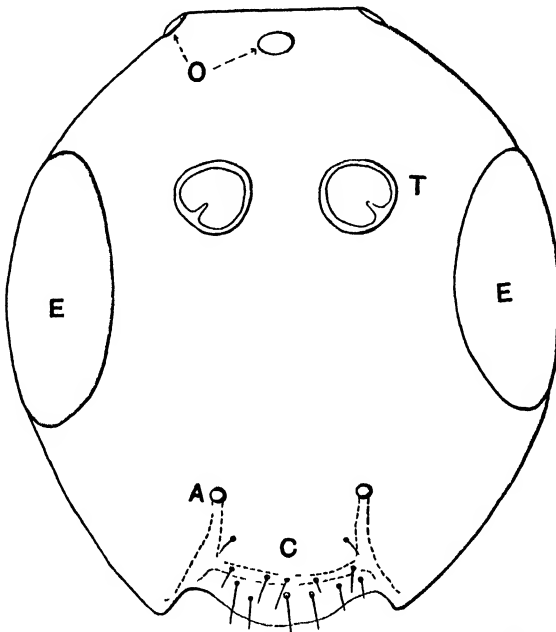


Fig. 5. *Rhoptromeris eucera*, female: outline of frontal aspect of head,  $\times 200$ . A, tentorial foramen (right); C, clypeus; E, compound eye; O, ocelli; T, torulus (left).

*Mouth-parts.* Mandibles (Fig. 6 C and D) equal sized, quadrate, average dimensions 0.1 mm.  $\times$  0.08 mm.; right mandible with three teeth, left usually with two teeth, more rarely a third tooth present. Maxillary palpi (Fig. 6 E) with joints related in length as 28:20:15 (measured along external border); terminal joint with an elongate external apical seta, a minute internal seta and a campaniform sense organ. Labial palpi (Fig. 6 E) 2-jointed, related in length as 15:17.

*Thorax.* Pronotum (Fig. 6 I) reduced to a small saddle-shaped shield less than one-third the breadth of the head; its anterior and posterior margins strongly raised leaving a transverse furrow between them; prosternum smooth and hairless. Mesonotum smooth with a scanty row of marginal hairs on either side; scutellum (Figs. 4 and 6 H) with two basal foveae and a very prominent median pyriform raised area which usually bears five short stout

setae (in rare cases two only); the detailed sculpture and chaetotaxy of the scutellum is variable; mesosternum hairy. Metanotum small and concealed by the projecting scutellum; metasternum densely hairy (Fig. 6 J). Propodeum (Fig. 6 J) with two dorsal longitudinal carinae, its ventral and side

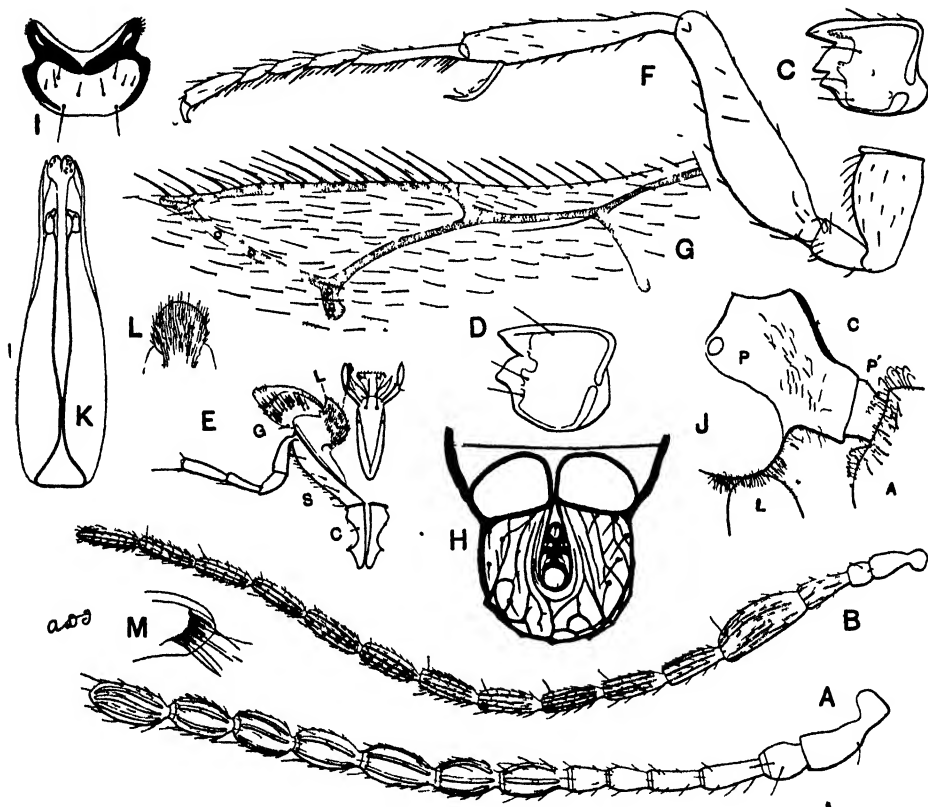


Fig. 6. *Rhoptromerus eucera*, structural features: B and K refer to the male, the remaining parts are those of the female. A, left antenna (female),  $\times 130$ . B, left antenna (male),  $\times 72$ . C, left mandible,  $\times 150$ . D, right mandible,  $\times 150$ . E, labium and right maxilla, ventral aspect,  $\times 65$ ; C, cardo; G, galea; L, lacinia; S, stipes. F, fore leg,  $\times 115$ . G, costal portion of left fore wing,  $\times 215$ . H, scutellum,  $\times 165$ . I, pronotum, dorsal aspect,  $\times 120$ . J, junction of thorax and abdomen, lateral aspect,  $\times 122$ : A, base of gaster; C, left dorsal carina of propodeum P; L, coxa of third leg; P, pedicel. K, male genitalia,  $\times 165$ . L, labrum, ventral aspect,  $\times 400$ . M, sensory plate of last abdominal tergum,  $\times 400$ .

walls hairy; spiracles circular. Fore wings (Figs. 4 and 6 G) 1.43 mm. to 1.76 mm. long and 0.60 mm. to 0.71 mm. maximum breadth. A conspicuous elongate macrochaeta at junction of sub-costal and radial veins<sup>1</sup>; rudiment of inter-cubital vein with four or more, rarely five, annuli; distal portion of radial vein with two annuli, very variable in position, being either adjacent to each

<sup>1</sup> The older vein nomenclature is followed in view of the difficulty in fixing the homologues under the Comstock-Needham system in wings with so greatly reduced and specialised venation.

other or separated by an interval; metacarpus with 12 conspicuous marginal macrochaetae<sup>1</sup>; traces of medius and cubitus faintly indicated. Hind wings 1.1 mm. long and 0.2 mm. broad; frenulum of three hooks, two annuli on the vein near by.

*Legs.* Fore legs (Fig. 6 F) with the coxa truncated proximally, length related to breadth as 11 : 7; femur bulging somewhat ventrally, related in length to tibia as 23 : 22; apex of tibia with a stout curved furcate ventral spur, 0.07 mm. long, and a minute dorsal peg-like spine; tarsal joints related in length as 11 : 5 : 4 : 3 : 6; the first tarsal joint with a ventral pecten of about 15 or 16 spines.

Middle legs are somewhat longer than fore pair; the coxa with proportions 15 : 8; femur related in length to tibia as 6 : 5; apex of tibia with a pair of ventral, slightly curved spurs of unequal length; tarsal joints as 11 : 6 : 5 : 3 : 6.

Hind legs with proportions of coxa as 20 : 9; femur shorter than the tibia, as 32 : 35; apex of tibia with a pair of ventral, slightly curved spurs of unequal length; tarsal joints as 16 : 6 : 5 : 4 : 7.

*Abdomen* (Fig. 6 J). Petiole hairless. Gaster smooth and shining with a conspicuous zone of irregular hooked hairs disposed around insertion of petiole and a ventral tooth below the latter. Last sternum prolonged into a simple point. Paired processes of last tergum each with a group of elongate sensory hairs as shown in Fig. 6 M.

Length (six examples) 1.4 mm. to 1.75 mm., wing-expanse 3 mm. to 3.75 mm.

#### *Description of the male.*

Shining pitchy black; antennae wholly black. Legs with coxae and trochanters black, their apices ochreous brown; femora and tibia black or brown-black with proximal and distal extremities ochreous; tarsi brown or brown-black, frequently darkening distally. Wings hyaline, veins fuscous.

The male can be readily separated from the female by the more elongate antennae (Fig. 6 B), which measure on an average 1.8 mm. long, and by the greatly enlarged fourth joint. The joints are mutually related in length as 10 : 5 : 11 : 17 : 12 : 13 : 12 : 12 : 12 : 12 : 12 : 12 : 14. The legs present no salient differences from those of the female except that the first tarsal joint is proportionately longer in all cases. The genitalia are shown in Fig. 6 K.

Average length 1 mm., wing-expanse 3 mm.

### III. *Loxotropa tritoma* Thoms.

The genus *Loxotropa* Thoms. belongs to the family Diapriidae whose members are essentially parasites of dipterous larvae. Its geographical distribution is very wide, species occurring over the greater part of Europe, in North America and the West Indies, and also in Java, New Guinea, the Seychelles, Philippines, Central Africa and Egypt. Only very scanty infor-

<sup>1</sup> In Fig. 6 G, there should be 12 instead of 14 marginal macrochaetae.

mation exists with reference to the host preferences of the genus, and there are more records of species living in association with ants than of rearings from individual hosts. In this connection it is noteworthy that species of the closely allied genus *Basalys* have also been described from ants' nests. There is no evidence that members of either genus are parasites of the ants and, in view of the general habit of the Diapriidae parasitising dipterous larvae, it appears probable that the myrmecophilous species are parasites of Diptera living in association with ants.

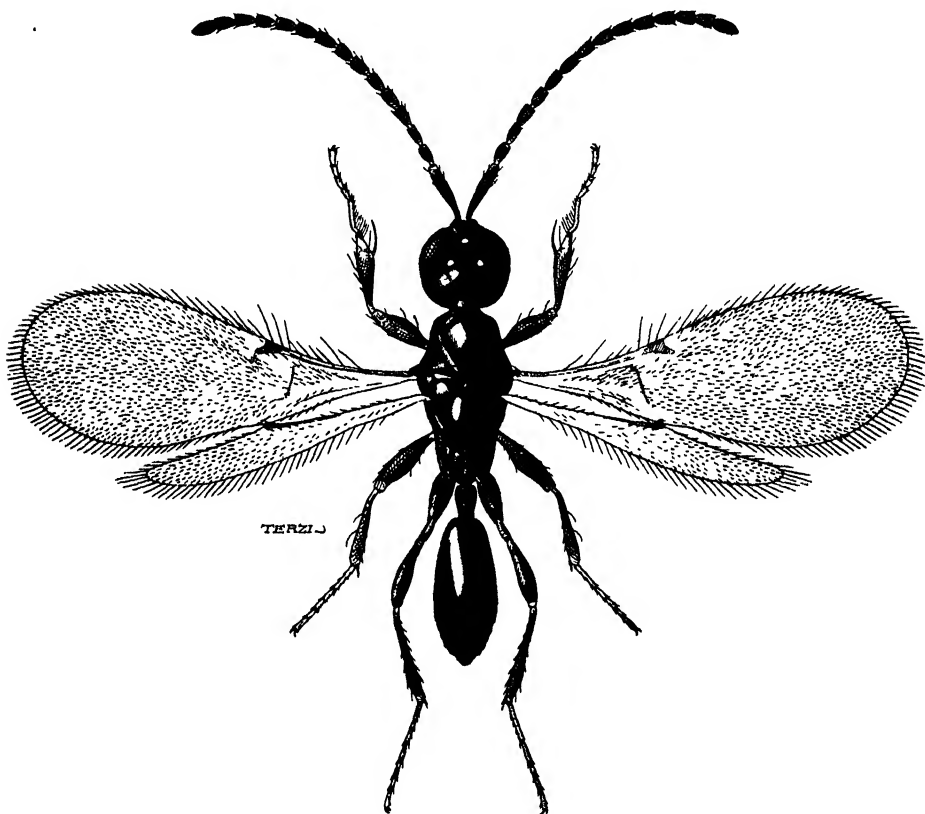


Fig. 7. *Loxotropa tritoma* Thoms., male,  $\times 36$ .

Donisthorpe (1927) enumerates the following species of *Loxotropa* as being found in ants' nests: *L. donisthorpei* Kieff., *formicarum* Kieff., *subterranea* Kieff., *fuliginosi* Box, *subregonensis* Box, and *tritoma* Thoms. Kieffer (1916) also enumerates *Loxotropa steueri* Kieff. from ants' nests in Cairo and *L. ashmeadi* Kieff. from nests of the bee *Halictus pruinosus* Rob. in Virginia, U.S.A., on the authority of Brues.

Among other records, *L. pegomyiae* Brues has been reared from *Pegomyia* (*Chortophila*) *brassicae* in Minnesota (Brues, 1907). *L. flavipes* Ashm. is mentioned by Bradley (1928) as a hyperparasite bred from cocoons of a Noctuid



moth, *Papaipema maritima* Edw., in New York State; and Kieffer (1916) records *L. hellicicola* Kieff. as being reared from a dipterous puparium found in a shell of *Helix aspersa* in France.

The first record of *L. tritoma* being a parasite of *Oscinella frit* is that of Meyer (1923) in Germany. Dr Cunliffe informs me that he reared four examples from that same host in the Oxford district in 1926 and the species has also been reared from *O. frit* by T. H. Taylor in the Leeds district. This insect does not appear to be an important parasite of frit-fly and in the Harpenden district only 15 examples were bred during 1926 and 1927, when they emerged between August 4th and August 23rd. Examples were reared by Dr Cunliffe on the following dates, July 20th-26th and August 11th, 1926; Meyer bred the insect in Germany in the month of July, Schander and Meyer record a single female on August 8th, 1923, and Thomson in his original description of the insect mentions August.

*Description of the female.*

*Coloration.* Shining black. Antennae brown-black or black, scape dark castaneous brown at its proximal and distal extremities; funicular joints dark castaneous brown, club dull black. Legs yellow-brown, femora fuscous, tibiae variably infuscated; fifth tarsal joint dark brown or black. Wings hyaline, apex of marginal vein of fore wing fuscous.

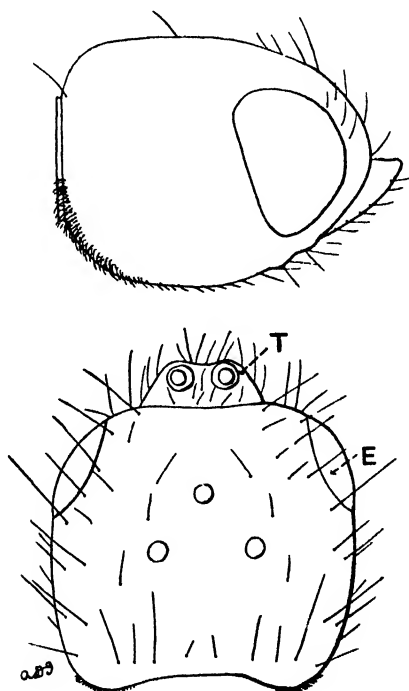


Fig. 8. *Loxotropa tritoma*, female. Above, outline of head, lateral aspect. Below, outline of head, dorsal aspect,  $\times 114$ . T, right torulus; E, compound eye. (All the setae are not shown.)

**Head.** Cuticle smooth, markedly bristly (Fig. 8). Viewed from above cuboidal in shape with the eyes placed at the anterior angles, 0.35 mm. long from hind margin to apex of frontal process, 0.30 mm. broad; viewed laterally the head is 0.25 mm. deep. Eyes small, widely separated and located very far forward; long axis 0.15 mm. Ocelli disposed as an equilateral triangle lying wholly on the vertex; median ocellus slightly in advance of line joining hind margins of eyes. Toruli circular, separated by about their own diameter. Labrum membranous and transparent, 0.05 mm. broad, armed with prominent setae as shown in Fig. 10 C. Occiput only slightly concave, clothed ventro-laterally with closely set, short, fine hairs.

**Antennae** (Fig. 9 A). Length 0.9 mm. to 1.0 mm.; scape 0.25 mm. long and 0.05 mm. broad, related in length to pedicel as 3 : 1. Funicular joints related respectively in length as 22 : 15 : 15 : 15 : 15 : 16; club equal in length to scape, its joints mutually related in length as 7 : 6 : 10. Sensoria present only on club; hairs on club become shorter and more numerous ventrally.

**Mouth-parts.** Mandibles (Fig. 10 A) of the two sides bidentate, 0.11 mm. long and 0.05 mm. maximum breadth. Maxilla as shown in Fig. 10 D, joints of palpi related in length as 8 : 8 : 5 : 6 : 8. Labial palpi 2-jointed; basal joint greatly narrowed proximally and swollen distally; apical joint ovate.

**Thorax.** Mesoscutum (Fig. 10 E) smooth and almost hairless, parapsidal furrows only defined anteriorly. Scutellum broadly T-shaped, strongly elevated, side walls hairy; axillae almost hairless; tegulae rather large. Metanotum bristly, band-like and of uniform breadth (Fig. 9 C); ornamented with three well-marked longitudinal carinae; inside the lateral carina there is a conspicuous macrochaeta on either side. Propodaeum (Fig. 9 D) with the tergal region strongly raised and with a median carina; all the margins with conspicuously raised borders; pleura hairy, spiracles circular. Posteriorly the propodaeum is produced into two stout backward processes separated by a deep semicircular sinus. Fore wing (Figs 7 and 9 B) hyaline, 1.4 mm. long and 0.5 mm. broad (including marginal hairs); marginal vein related in length to wing as 11 : 28, its apex with four minute and obscure annuli; basal vein not joined with marginal. Beyond the basal vein the wing-membrane is uniformly invested with sub-equal hairs except for a small hairless tract adjacent to the apex of the marginal vein. Hind wing 1.05 mm. long and 0.22 mm. maximum breadth; marginal vein vestigial, only clearly evident at proximal and distal extremities. Frenulum of two hooks, preceded by a slender recurved seta and flanked by a pecten of 8 or 9 setae.

**Legs.** Fore leg (Fig. 10 F) with coxa small and globular, its length only slightly exceeding the diameter. First joint of trochanter slender, exceeding coxa in length. Femur (excluding second joint of trochanter) approximately equal in length to tibia, distinctly inflated. Tibia clavate, its length related to maximum breadth as 17 : 5. Tarsal joints related in length as 9 : 3 : 2.5 : 2 : 4, first joint markedly curved and with a pecten of closely set spines; apical spur 0.1 mm. long, bifid and ciliated; claws bristly on their bases.

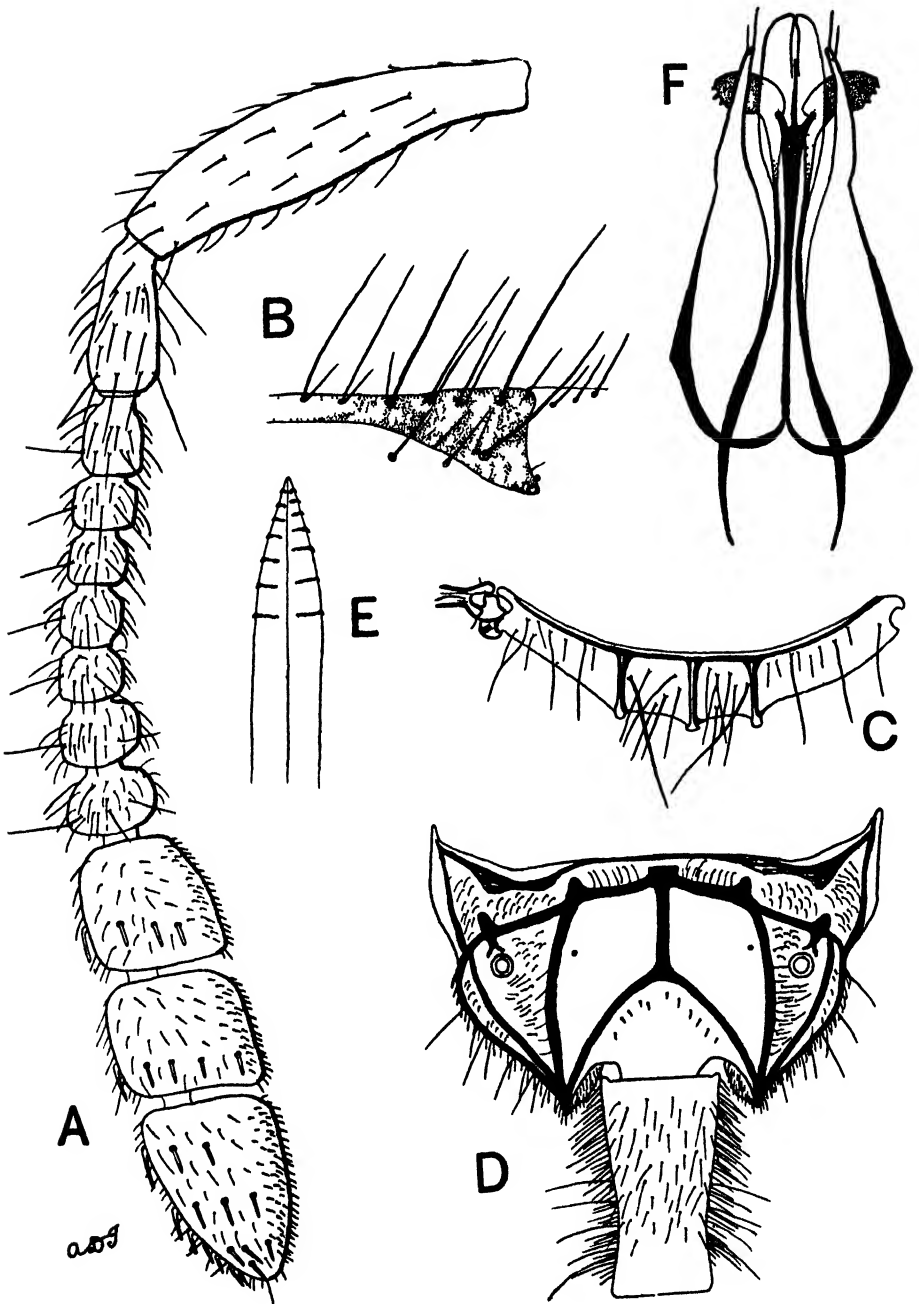


Fig. 9. *Lozotropa tritoma*, structural features: excepting F, the parts are those of the female. A, antenna (right)  $\times 220$ . B, apex of marginal vein of right fore wing,  $\times 380$ . C, metanotum, dorsal aspect,  $\times 155$ . D, propodeum and pedicel, dorsal aspect,  $\times 155$ . E, apex of ovipositor,  $\times 900$ . F, male genitalia,  $\times 400$ .

Middle leg with coxa rather more elongate than fore coxa, its length related to the breadth as 3 : 2. First joint of trochanter slender, equal in length to coxa. Femur slightly shorter than tibia, its length related to maximum

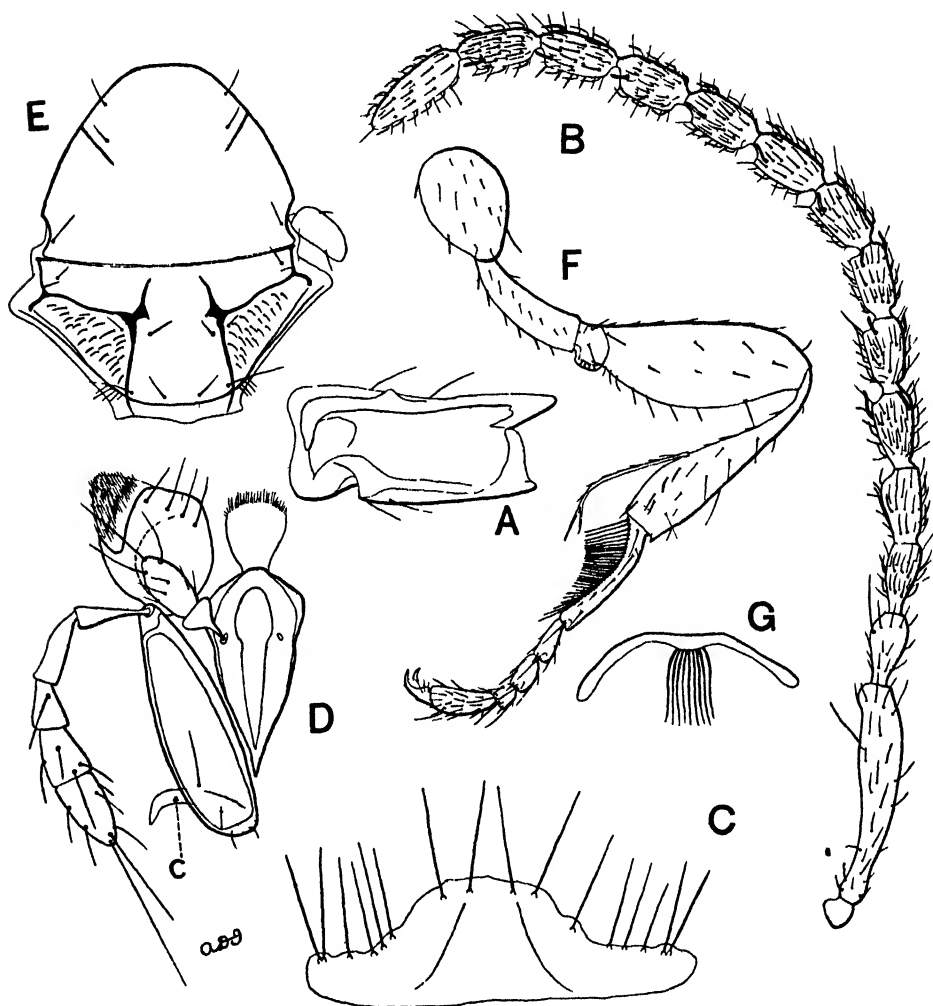


Fig. 10. *Lozotropa tritoma*, structural features: excepting B the parts are those of the female. A, mandible (right),  $\times 345$ . B, right antennae (male),  $\times 115$ . C, labrum,  $\times 900$ . D, labium and right maxilla,  $\times 250$ ; C, cardo. E, pronotum and mesonotum,  $\times 130$ . F, right fore leg,  $\times 145$ . G, margin of dorsal wall of sinus at base of first tergum of gaster, showing median group of setae,  $\times 312$ .

breadth as 17 : 6. Tibia clavate, its length related to maximum breadth as 5 : 1; two small apical spurs of unequal length. Tarsal joints related in length as 12 : 7 : 7 : 6 : 12.

Hind leg with coxa more elongate than that of second leg, its length related to the breadth as 17 : 7. First joint of trochanter markedly shorter than coxa.

Femur somewhat inflated, distinctly shorter than the tibia. Tibia with length related to breadth as 33 : 5, apical spur straight, 0.06 mm. long. Tarsal joints related in length as 20 : 11 : 10 : 8 : 14.

*Abdomen.* Pedicel 0.18 mm. long and 0.1 mm. maximum breadth; densely setose laterally, much less so dorsally and ventrally. First segment of gaster with a small sinus for reception of petiole; dorsal wall of this sinus has a strongly chitinised margin and bears a median downwardly directed tuft of regularly arranged setae of about equal length (Fig. 10 G). Apices of stylets of ovipositor each with seven minute serrations. The general surface of the gaster is smooth and for the most part hairless.

Length 2 mm., wing-expanse 3 mm.

#### *Description of the male (Fig. 7).*

In general coloration it is similar to the female. The only notable secondary sexual differences are seen in the shape of the head and the form of the antennae. The head is slightly broader than long, the breadth being related to the length as 35 : 32. The mean length of the antennae (Fig. 10 B) is 1.5 mm.; scape 0.22 mm. long, related in length to pedicel as 22 : 9; remaining joints, except the last, sub-equal in length, each joint being either equal to, or slightly longer than, the pedicel; terminal joint more elongated and rather more than half the length of the scape. In addition to ordinary hairs the flagellum bears curiously bent and forwardly directed setae resembling the sensoria, but of a much more slender form; sensoria are present on each joint of the flagellum excepting the first two joints, two sensoria being visible on each joint when viewed laterally. The relative proportions of the tarsal joints of the three pairs of legs are 12 : 4 : 3 : 3 : 6, 10 : 5 : 5 : 4 : 7, and 14 : 7 : 6 : 5 : 8. The genitalia are shown in Fig. 9 F.

Length 1.5 mm., wing-expanse 3.25 mm.

#### 4. REMARKS ON THE PARASITISM.

Observations made during 1926 and 1927 indicate that frit-fly in England is attacked by at least two species of parasites of considerable significance, viz. *Rhoptromeris eucera* and *Halticoptera fuscicornis*. These two species were also reared by Messrs Cunliffe and Taylor in the Oxford and Leeds districts respectively. Other species of apparently minor significance have been reared and among them the Proctotrypid *Loxotropa tritoma* is noteworthy. In Figs. 11 and 12 the total rearings of host and parasites are expressed graphically. The method of investigation adopted is too laborious to admit of the rearing of very large numbers of individuals or to allow of far-reaching conclusions to be drawn; nevertheless, the data obtained indicate that the host is evidently controlled by parasites to a very considerable degree.

During 1926 (Fig. 11) 27 per cent. of the insects reared were parasites which numbered 84 individuals as follows. *Rhoptromeris eucera* 24 males,

18 females; *Halticoptera fuscicornis* 9 males, 28 females; *Loxotropa tritoma* 2 males, 3 females.

During 1927 (Figs. 12, 13), when three plots were laid down, 37 per cent. of the individuals reared were parasites, viz. *Rhoptromeris eucera* 78 males,

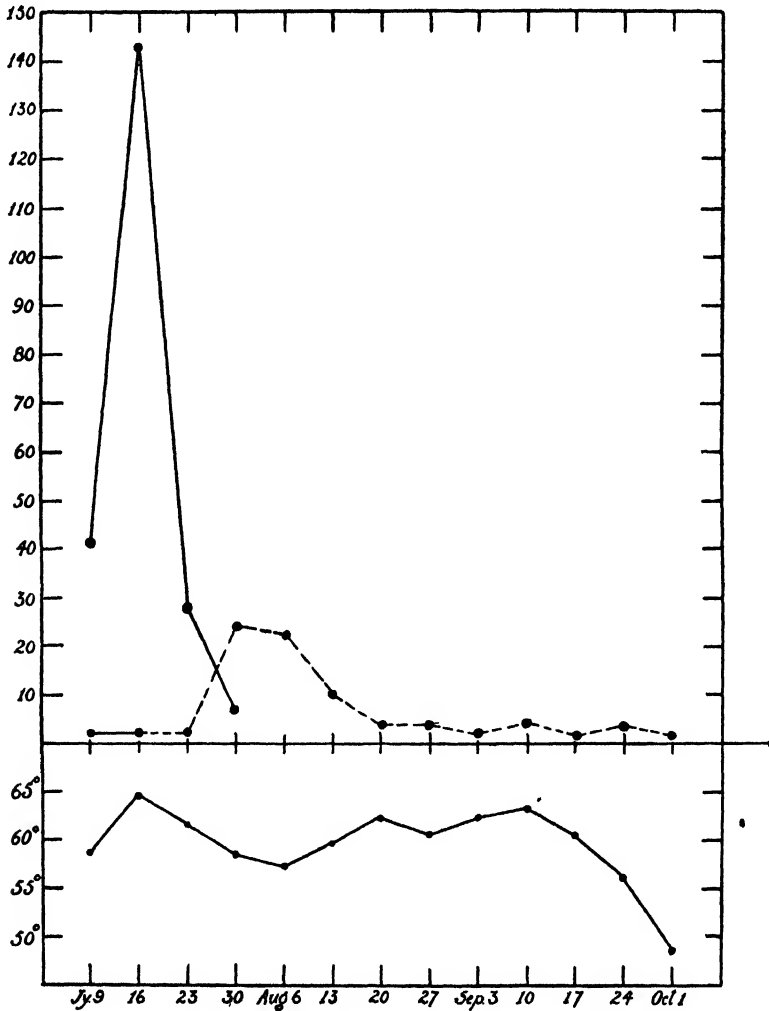


Fig. 11. Number of individuals of *Oscinella frit* and parasites (broken line), which emerged during each seven-day period at Harpenden, 1926: the first period ended on July 9th and the last period ended on October 1st. Below is shown the weekly mean temperature (°F) during that time.

70 females; *Halticoptera fuscicornis* 11 males, 29 females; *Loxotropa tritoma* 4 males, 6 females. Records of parasites reared from the plots taken individually are as follows (Fig. 13). Plot I. *H. fuscicornis* 3 males, 1 female; *R. eucera* 11 males, 11 females; *L. tritoma* 0. Plot II. *H. fuscicornis* 2 males, 16 females; *R. eucera* 36 males, 26 females; *L. tritoma* 2 males, 4 females.

Plot III. *H. fuscicornis* 6 males, 12 females; *R. eucera* 31 males, 33 females; *L. tritoma* 2 males, 2 females.

As already mentioned on p. 2 the parasitism of the host affecting the respective plots increased from 15 per cent. on Plot I to 40 per cent. on Plot II and to 54 per cent. on Plot III. This would appear to indicate that the parasites become progressively more numerous as the season advances, with the result that frit-fly, affecting late sown oats, is markedly heavier parasitised than

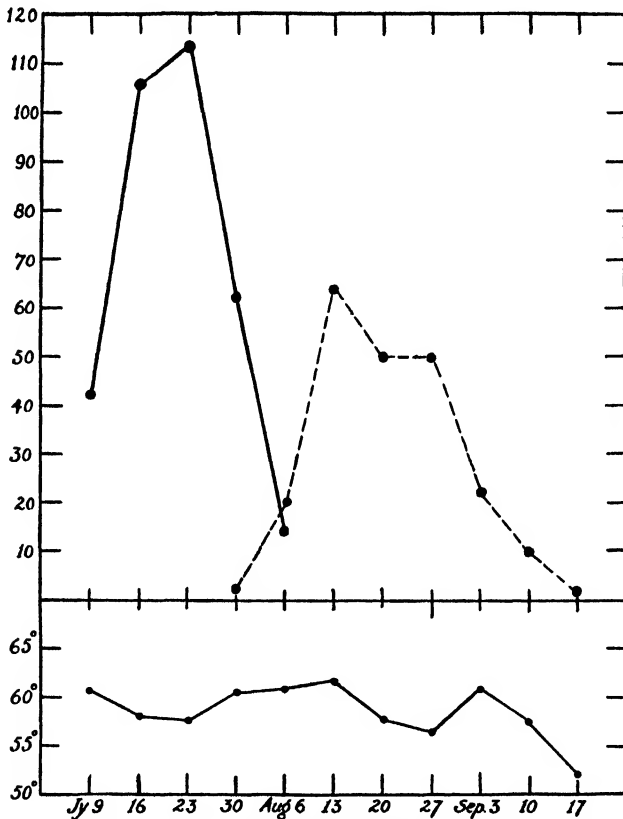


Fig. 12. Number of individuals of *Oscinella frit* and parasites (broken line), which emerged during each seven-day period at Harpenden, 1927: the first period ended on July 9th and the last period ended on September 17th. Below is shown the weekly mean temperature (°F) during that time.

when it attacks oats drilled earlier in the season. In so far as my observations extended, only a single individual parasite emerged from a host puparium and consequently each parasite represents the destruction of an individual host.

Schander and Meyer (1925) record the numbers of host and parasites reared in Germany between July 8th and August 9th, 1922. In Fig. 14 their figures have been expressed as a graph and the percentage of parasites reared

was 31.7, which is almost exactly the mean of the parasitism recorded in the present paper for the years 1926 and 1927.

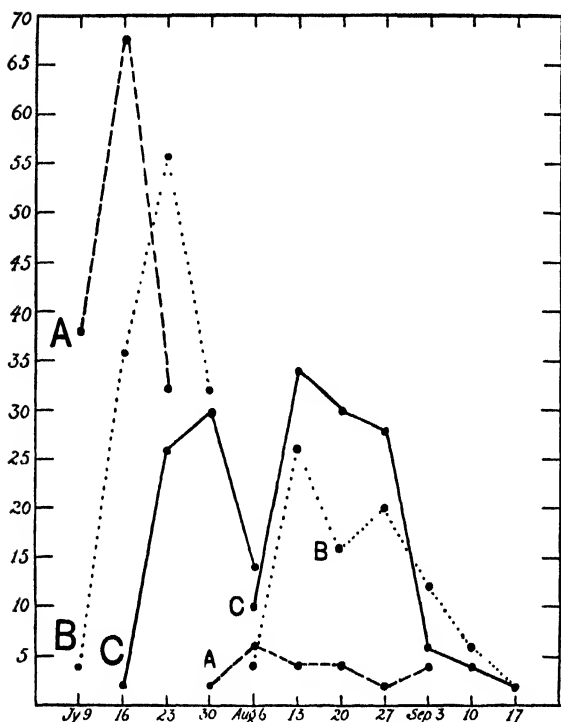


Fig. 13.

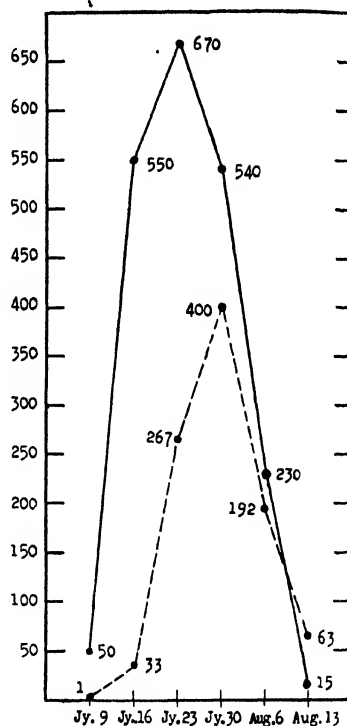


Fig. 14.

Fig. 13. Number of individuals of *Oscinella frit* and parasites, which emerged during each seven-day period at Harpenden, 1927: the first period ended on July 9th and the last period ended on September 17th. A, host and  $\Delta$ , parasites emerging from oats sown on March 15th; B,  $\Delta$ , the same for oats sown on March 26th; C,  $\Delta$ , the same for oats sown on April 25th.

Fig. 14. Number of individuals of *Oscinella frit* and parasites (broken line), which emerged during each seven-day period at Landsberg a.W. (Germany), 1922: the first period ended on July 9th and the last ended on August 13th. Based upon figures given by Schander and Meyer (1925).

The following is a list of the parasites recorded as utilising *Oscinella frit* as a host. It must be pointed out that in certain cases the records are doubtful and consequently need confirmation. The most important parasites are indicated thus \*.

## CYNIPOIDEA.

### FIGITIDAE.

#### 1. *Cothonaspis* (Sub-gen. *Hexaplasta*) *hexatoma* Först.

(*Hexaplasta fuscipes*, Meyer, 1923.)

Germany: Meyer, 1923, 1924; Hedicke, 1923.



- \*2. *Eucoila* (Sub-gen. *Rhoptromeris*) *eucera* Htg.  
(*Rhoptromeris widhalmi* Kurd. 1912.)  
Germany: Meyer, 1923, 1924; Hedicke, 1923. Russia: Baranov,  
1912. England: Cunliffe; Imms.

## CHALCIDOIDEA.

## MISCOGASTERIDAE.

- \*3. *Halticoptera fuscicornis* (Walk.).  
(*Dicyclus fuscicornis* Walk. 1833.)  
England: Cunliffe, 1921; Imms.
4. *Halticoptera petiolata* Thoms.  
Germany: Meyer, 1923; Schander and Meyer, 1925.
- \*5. *Halticoptera suilius* (Walk.).  
(*Miscogaster suilius* Walk. 1833: *Pachylarthrus suilius* Walk.  
1846.)  
Germany: Meyer, 1923, 1924; Schander and Meyer, 1925.  
It appears doubtful whether this species is one distinct from  
*H. fuscicornis* and Dr Waterston informs me that the name *suilius*  
may prove to have been given to the male of *H. fuscicornis*.
6. *Semiotellus nigripes* Lind.  
Russia: Wilhelm, 1891.

## PTEROMALIDAE.

7. *Stenomalus micans* (Ol.).  
(*Pteromalus micans* Ol.)  
Russia: Rorig, 1893; Schesterikov, 1910.
8. *Pteromalus puparum* L.  
Russia: Rorig, 1893.
- \*9. *Trichomalus cristatus* (Först.).  
(*Pteromalus cristatus* Först. 1841; *Trichomalus frontalis* Thoms.  
1878.)  
Russia: Baranov, 1912. Germany: Meyer, 1923, 1924; Schander  
and Meyer, 1925.
10. *Merisus intermedius* Lind.  
Russia: Wilhelm, 1891.
11. *Polycystus oscinidis* Kurd.  
Russia: Mokrzecki, 1913.
12. *Callitula bicolor* Spin.  
England: Imms. The first record from this host.

## EULOPHIDAE.

13. *Neochrysocharis immaculatus* Kurd.  
Poland: Kurdjumov, 1912.

## MYMARIDAE.

14. *Gonatocerus sulphuripes* (Först.).  
 (*Rachistus sulphuripes* Först. 1847.)  
 Germany: Schander and Meyer, 1925.

## ICHNEUMONOIDEA.

## BRACONIDAE.

15. *Sigalphus caudatus* Nees.  
 England: Curtis, 1860. Italy: Silvestri and Grandi, 1911. Russia:  
 Rorig, 1893.
16. *Gyrocampa pospelovi* Kurd.  
 Russia: Kurdjumov, 1912. Germany: Schander and Meyer, 1925.
17. *Chasmodon apterus* (Nees).  
 (*Alysia aptera* Nees et al.)  
 England: Cunliffe, 1921.
18. *Aphidius granarius* Marsh.  
 England: Cunliffe, 1921.

## PROCTOTRYPOIDEA.

## DIAPRIIDAE.

19. *Loxotropa tritoma* Thoms.  
 England: Cunliffe; Imms. Germany: Meyer, 1923; Schander and  
 Meyer, 1925.

It is noteworthy that no observations have been recorded in North America respecting the parasitisation of frit-fly on that continent. According to Aldrich (1920), this insect "appears to be freely parasitised by minute Hymenoptera, but observations have not as yet excluded all doubt in any case."

During 1926 the mean temperature over the period covered by the observations was markedly higher than for the corresponding period in the following year. The maximum emergence of frit-fly in rearing cages took place during the seven-day period ending July 16th. This fact is of considerable interest in relation with field observations made by Cunliffe, who states that during the last six years the second generation of flies appeared in its maximum numbers about July 15th each season (Cunliffe, 1929). During 1927 the mean temperature at Harpenden was lower than in 1926, but this feature appeared to have little effect upon the time of emergence of the flies, as will be seen from Fig. 13. In this connection reference to Fig. 14 shows how closely the period of maximum emergence of flies reared by Schander and Meyer in Germany is in accordance with Cunliffe's field observations in England.

## 5. SUMMARY OF CONCLUSIONS.

The stem infestation of *Oscinella frit* in England is attacked by at least two species of hymenopterous parasites of considerable importance. The species are *Halticoptera fuscicornis* Walk. (Chalcidoidea) and *Rhoptromeris eucera* Htg. (Cynipoidea). It is also parasitised to a much lesser degree by *Loxotropa tritoma* Thoms. (Proctotrypoidea). These three species are described and figured in detail.

The three species named above have been reared by the writer from host puparia obtained in the Harpenden district; they have also been reared in the Leeds and Oxford districts by other observers. They attack the host in the larval stage of the latter and emerge from the puparia. All three species are characteristic parasites of cyclorrhaphous Diptera.

In the Harpenden district a parasitism of 27 per cent. was observed in 1926 and of 37 per cent. in 1927, *Rhoptromeris eucera* being the dominant parasite.

Evidence is brought forward which appears to indicate that the parasites, collectively, become more abundant as the season advances, with the result that *Oscinella frit* affecting late sown oats suffers markedly heavier parasitisation than when it attacks oats drilled earlier in the season.

The time of maximum emergence of *Oscinella frit* in out-of-door rearing cages during the years 1926 and 1927 coincided very closely with its period of greatest abundance in the field, as recorded by Cunliffe.

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# ON THE STRUCTURE OF THE IMMATURE STAGES OF THE FRIT FLY (*OSCINELLA FRIT* LINN.)

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(With 11 Text-figures.)

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## I. INTRODUCTORY REMARKS.

THE following paper deals with experiments and descriptions concerning the biology and structure of the immature stages of *Oscinella frit*, the frit fly of oats. The work has been carried out at the Rothamsted Experimental Station under the direction of Dr A. D. Imms.

A complete list of literature relating to *Oscinella frit* is not given because a summary of our knowledge of this insect, up to 1918, is provided by Collin(2). He includes (in his bibliography) thirty-three references to papers in addition to several full references given in the text. Since 1918 extensive investigations into the habits, prevalence and damage caused by *Oscinella frit*, and the possible use of resistant varieties of oats, have been carried out in England by Cunliffe, Fryer and others. In America Aldrich(1), in 1920, contributed a paper on the biology and habits of the European frit fly on wheat in that country. Frit fly attack in the stems of cereals is usually recognisable by the nature of the damage which is caused, viz. the central shoot usually dies and turns

brown whilst the surrounding shoots remain green. This is, however, not invariably the case, and where the characteristic signs of attack are missing it is necessary, for diagnostic purposes, to examine the morphology of the larva. Up to the present time no adequate description of any of the larval stages has appeared, and it is in an attempt to supply this deficiency that the present work has been carried out.

### *Technique.*

The experiments were conducted in an outdoor insectary, which was fitted with glass at the front and top, whilst wire netting at each end allowed free circulation of air through the house; hence the experiments, whilst protected from rain, obtained as much sunlight as possible, and also a good supply of air.

For the purpose of rearing larvae, to work out the different stages in the life history, pots 11 inches in diameter containing insect-free soil were used. Oat seeds, variety "Supreme," were sown in two rows of six seeds each; the seeds in each row were approximately an inch apart, and the rows 2 inches apart, in order that the eggs might be detected without difficulty. The pots were protected with cages whose sides were covered with cellophane and the tops with muslin. Cellophane was found to be preferable to glass, since, while allowing the maximum entry of light, there was no condensation of moisture on the surface inside. The muslin-covered top gave access to the air.

In order to infect the experimental plants, mature insects were collected in tubes, in the numbers required, and introduced into the cages. In all experiments a small dish, containing sugar solution, was placed in each cage to provide nourishment for the insects.

To obtain first stage larvae, eggs were taken from the experimentally infected plants and placed on a piece of leaf blade, upon moistened filter paper, in a glass square. On hatching the young larvae bored into the leaf blade and were obtained by dissecting them out, under a binocular microscope. In order to investigate the tracheal system of the first stage larvae, very young individuals were taken which had not yet started to feed. These were obtained by placing eggs, in which the movements of the larvae were visible, into a moistened glass square. If moist filter paper is used the larvae, on emergence, burrow into it, and, being exceedingly small and almost transparent, are very difficult to detect. The tracheal system of all three larval stages was mapped out by placing living larvae, along with a small quantity of water, under a coverslip and gently pressing the latter.

*Notes on the biology of Oscinella frit.*

From the experiments that were set up to provide material for the study of the structure of the developmental stages, a number of general observations were made. These were supplemented by data obtained on the Rothamsted farm plots. In the experimental cages the eggs, in the majority of cases, were deposited inside the sheath at the base of the stem. The female insect protrudes the terminal segments of the abdomen and inserts the eggs within the fairly closely adhering sheath. In a few instances eggs were laid on the outside of the stem close to the surface of the earth; in the first leaf sheath or, rarely, on the base of a leaf blade. The number of eggs laid inside each sheath varied from one or two up to—in a few cases—thirteen, fourteen or fifteen, usually in groups. The majority of eggs kept under observation hatched in 3-4 days, but during colder periods some of them took 6-7 days. In the few cases where the eggs were laid on the bases of leaf blades, the larvae upon hatching mined through the mesophyll of the leaf in order to reach the shoot. Experiments carried out with plants at different stages of growth indicate that the heaviest infestation occurs in the two- and three-leaf stages. Eggs, however, were laid in moderate numbers on plants in the four- and five-leaf stages, but older plants were, with a few exceptions, neglected. Occasionally older plants, although not showing the characteristic external indication of attack, were found on dissection to contain larvae.

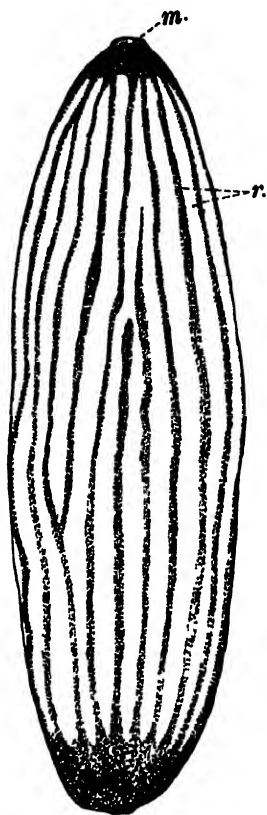


Fig.1. Egg.  $\times 155$ . *m.* micropylar area; *r.* longitudinal ridges.

## II. THE EGG (Fig. 1).

The eggs are fusiform in shape, slightly curved and taper to each end. The posterior extremity is broadly round whilst the tapering is much more marked at the anterior end. The eggs vary in length from 0.58 mm. to 0.73 mm. with an average of 0.68; the maximum width ranges from 0.13 mm. to 0.20 mm. with an average of 0.16 mm. The colour, as seen with the naked eye, is glistening white, but appears creamy white when



examined in reflected light under the microscope. The surface of the egg is sculptured into a number of ridges and grooves which run roughly in a longitudinal direction; many of the grooves extend the whole length of the egg, whilst some bifurcate and a few end abruptly. At the posterior pole the surface is ornamented by a number of small polygonal areas. When examined under the high power of the microscope the ridges appear as long rows of closely apposed, bead-like papillae of thickened chorion, whilst the grooves are occupied by the softer portions of the chorion and have large numbers of papillae distributed over them.

In a median position, at the tapered anterior pole, there is a slight constriction which gives the region in front of it a cup-like appearance, when seen in surface view. The inside edges of the cup are directed backwards and inwards towards the constricted region, the centre of which is occupied by a depressed area of thinner chorion. The almost flat, posterior pole is strengthened by a circular area of thickened chorion.

#### *Emergence of the larva.*

The larva emerges through an irregular longitudinal slit at the anterior pole, slightly to the outside of the cup-like area. Wriggling movements of its body, and the scraping of the mouth hooks on the chorion, are continued for several hours before the larva finally emerges from the egg. This object is attained by the scraping action of the mouth hooks, facilitated by pressure produced by the movements of the enclosed larva, causing a fairly large slit to be made. The chorion, after the emergence of the larva, assumes a flattened, shrunken appearance and the exit opening is noticed as a roughly V-shaped slit.

### III. THE LARVAL INSTARS.

Three definite larval instars were observed, each showing, on careful examination, marked differences from the other. The main differences, apart from that of size which is not a sure indication (especially about the time of each ecdysis), are seen in the cephalo-pharyngeal skeleton and the spiracles. These are figured and described in a later part of this paper. The first stage larva has been taken in the act of emergence from the egg. Specimens have been obtained showing, respectively, the second stage larva still enclosed in the cuticle of the first stage; the third stage larva enclosed within the cuticle of the second stage; and also the cuticle of the third stage larva hardening to form the puparium. It will be evident, therefore, that errors in the determination of the respective instars have been avoided.

*(a) The first instar larva.*

The first instar larva varies in length from 0.7 mm. to 1.5 mm. with an average of 1.05 mm.; the maximum width ranges from 0.13 mm. to 0.23 mm. with an average of 0.16 mm. It is cylindrical in shape, rounded at the posterior end and tapering slightly towards the anterior end. It is almost transparent with the cuticle smooth, except at the junctions of the segments.

*The head*, which is somewhat retracted, is divided by a median depression into two symmetrical parts. It bears anteriorly a pair of two-jointed antennae; the basal joint is short and into its apex fits the globe-shaped second joint. Slightly posterior to these, and on the ventral surface, occur the maxillary palpi which arise as prominences from the integument. The maxillary palp consists of a group of rounded papillae of different sizes, each of which, in surface view, appears as a small ring with a dark central region; the group of papillae is bounded on its inner, posterior, and part of the outer margin, by a dark chitinous ridge. Between the anterior lateral margin of each maxillary palp, and the median depression, there is situated a single sensory papilla. Posterior to each maxillary palp, and on each side of the median depression, there is a ventral sensory organ. This structure consists of a chitinous ring in the centre of which are two adjacent rounded papillae, with a smaller one below them; the papillae are similar to those of the maxillary palp. The maxillary palp and ventral sensory organ, of each side, is enclosed within a long, oval-shaped area. A toothed chitinous plate, similar to that figured by Keilin (4) and de Meijere (5) for *Calliphora erythrocephala*, occurs on the integument at the right and left anterior margins of the mouth opening. According to Keilin this paired structure acts as a grater, and assists the buccal armature in lacerating the tissues of the host; in the first stage larvae of *Pegomyia winthemi*, where the buccal armature is feebly developed, these are strong hooks united to form a single grater (*vide* Keilin (4)). At the posterior border of the mouth, and close to the median line, is a pair of papillae.

*The cephalo-pharyngeal skeleton* (Fig. 2) is composed of paired sclerites; the most anteriorly situated of these are the mouth hooks whose apices project from the mouth opening. The mouth hooks are brownish yellow in colour and each has three sharply pointed teeth on its inner edge; the apical tooth is considerably larger than the other two which are similar in size and shape. Posteriorly to each mouth hook, and attached to it, is a long narrow accessory sclerite (*a.s.*); to its lower ventral surface is

articulated a second accessory sclerite in the form of a rounded nodule. Posterior to this latter sclerite, and articulated with it, is the intermediate or hypostomal sclerite which is completely fused with the basal or pharyngeal sclerite. The intermediate sclerite, though dark at its anterior end, becomes gradually paler and less sclerotised towards the distal extremity. It appears as an anterior process which broadens out

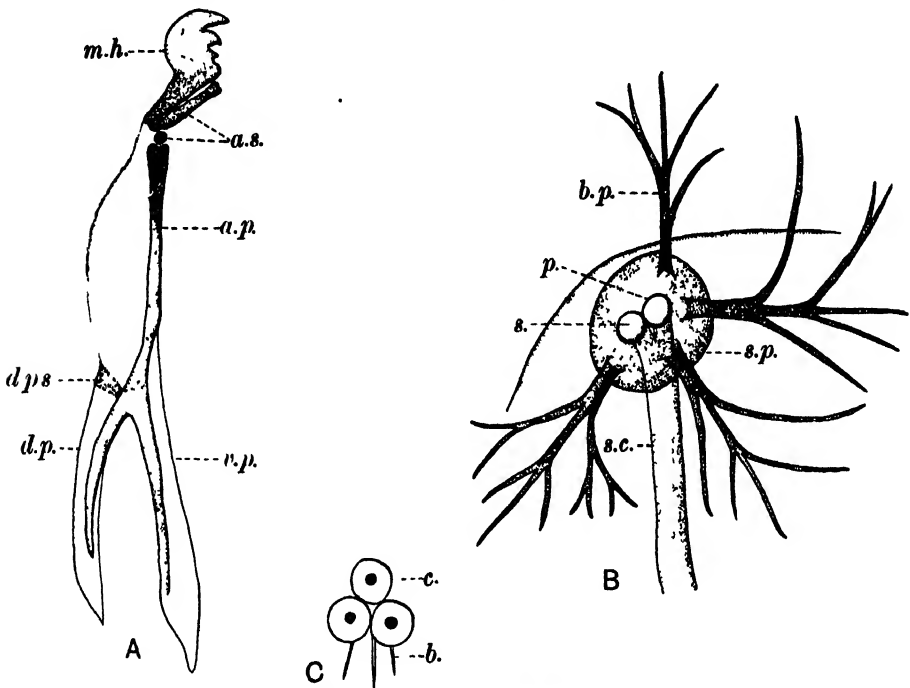


Fig. 2. A. Cephalo-pharyngeal skeleton of first larval instar  $\times 375$ . *a.p.* anterior process of pharyngeal sclerite; *a.s.* accessory sclerites; *d.p.* dorsal process of pharyngeal sclerite; *d.p.s.* remnant of dorsal pharyngeal sclerite; *m.h.* mouth hook; *v.p.* ventral process of pharyngeal sclerite.

B. Posterior spiracle of first larval instar (surface view).  $\times 1120$ . *b.p.* branched process; *p.* peritreme; *s.* spiracular opening; *s.c.* stigmatic chamber; *s.p.* stigmatic plate.

C. Organ on ventral surface of thoracic segments. Highly magnified. *b.* minute bristle; *c.* circle of cuticle.

posteriorly and passes into the pharyngeal sclerite; this sclerite bifurcates to form the dorsal and ventral processes, the latter being the longer. The anterior process (intermediate sclerite) isolates itself in the second stage larva as is the case with several other Dipterous larvae. The area (*d.p.s.*) which lies dorsal to the pharyngeal sclerite, near the region of bifurcation, is probably a vestige of the dorsal pharyngeal sclerite which, in some species, unites the anterior ends of the pharyngeal sclerites.

**Body segments.** There are eleven body segments, three thoracic and eight abdominal. The lines of junction of the segments are marked by circular swellings which are most prominent on the ventral surface; these, with the exception of the first two, are surrounded by transverse rows of minute cuticular denticles which are least numerous on the third segment. The rows, which are not continuous but occur in sets, are numerous on the ventral surface but gradually diminish in numbers towards the dorsal surface. The margin of the head and first thoracic segment is also surrounded by numerous rows of minute denticles. The thoracic denticles are long and pointed posteriorly, whilst those which occur on the abdominal segments have rounded apices.

About the middle of the ventral surface of each thoracic segment, and on each side of the median line, is an organ composed of three closely apposed circles (Fig. 2 C). The circles are arranged with one to the right, one to the left and the third behind, and each bears a minute seta. Keiln(4) has found similar organs, only differing in detail, in all Dipterous larvae which he has examined and, as they are in direct relation with the imaginal discs of the legs, maintains that they are vestiges of the ancestral larval thoracic feet. This interpretation has also been adopted by Pérez(6) in apodous Coleopterous larvae.

In a median position on the ventral surface of the eighth abdominal segment is a longitudinal slit, the anus; it is bordered on either side by a semi-circular raised area. The whole is surrounded by a ring of chitin.

**Tracheal system.** The first stage larva, like that of most *Cyclorrhapha*, is metapneustic and there is no indication of anterior spiracles (Fig. 3). The posterior spiracles occur at the apices of rounded prominences which arise as prolongations of the last abdominal segment. The openings of the spiracles are two in number, circular in shape, and each is surrounded by a ring of stiff chitin forming the peritreme. The spiracular openings lead through separate channels into the stigmatic chamber which, in turn, is in communication with the main tracheal trunk. The stigmatic

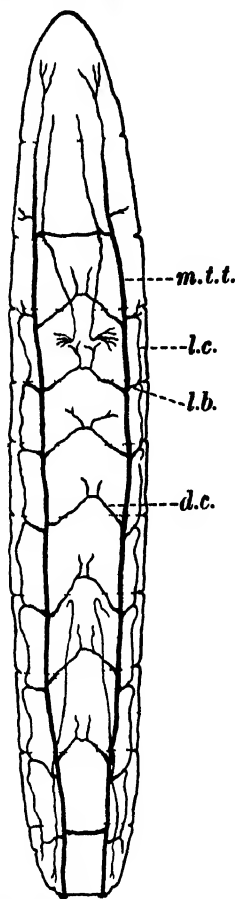


Fig. 3. Tracheal system of first larval instar  $\times 107$ . *d.c.* dorsal commissure; *l.b.* lateral branch; *l.c.* lateral commissure; *m.t.t.* main tracheal trunk.

chamber may be distinguished from the adjoining trachea by the granular texture of the walls and the absence of spiral thickening. Situated laterally are four chitinised, hair-like structures each with a few branches. Seen in side view they have the appearance of supports to the membrane surrounding the spiracles.

In the first stage larva the main tracheal trunks run dorsally from the posterior spiracles along each side of the body, to terminate in fine branches in the first thoracic segment. The two trunks are connected by a dorsal commissure in each segment excepting the first. The first and last dorsal commissures are, however, much stouter than the others and

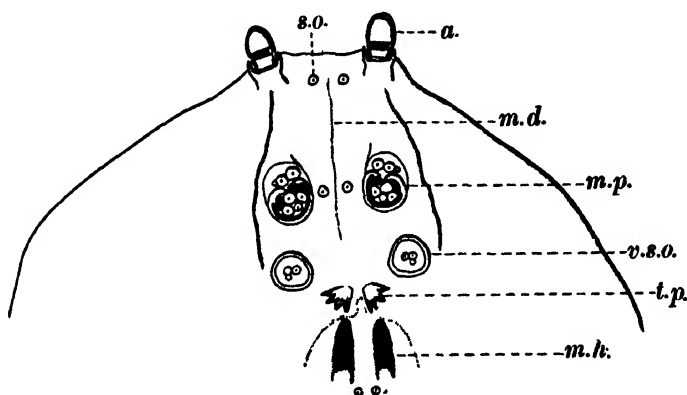


Fig. 4. Ventral view of head of second larval instar.  $\times 560$ . *a.* antenna; *m.d.* median depression; *m.h.* apices of mouth hooks; *m.p.* maxillary palp; *s.o.* sensory organ between antennae; *t.p.* toothed plate; *v.s.o.* ventral sense organ.

run straight across from one longitudinal trunk to the other. The remaining commissures are slender and each makes a loop into the preceding segment; near the middle of each loop two small branches are given off anteriorly. Lateral branches are given off also from the main trunk, of which all but the first three are connected by a series of longitudinal commissures. From the latter, branches run out to the body wall.

#### (b) *The second instar larva.*

The second instar larvae vary in length from 1.7 mm. to 2.5 mm. with an average of 2.04 mm.; the maximum width ranges from 0.2 mm. to 0.4 mm. with an average of 0.28 mm. It is similar in shape to the first stage larva and, seen with the naked eye, is scarcely distinguishable from it. The head (Fig. 4) bears a pair of antennae, maxillary palps and ventral sensory organs, all of which are similar in structure to those of the

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preceding stage. Situated between the antennae, and slightly posterior to them, is a pair of sensory papillae, one on each side of the median line. Toothed chitinous plates also occur at the anterior-lateral margins of the mouth opening.

*The cephalo-pharyngeal skeleton* (Fig. 5 A). The mouth hook has a large apical tooth; between this and the two posterior teeth is a small one which is not found in the first stage larva. The elongate accessory sclerite has undergone degeneration and become fused with the mouth hook; it is seen as a slight projection from the posterior ventral edge of the mouth hook. Immediately below the median projection from the ventral surface of the mouth hook is a small arc-shaped piece; this is probably the dentate sclerite to which is attached the mandibular depressor muscle. In this stage the anterior arm of the pharyngeal sclerite of the first stage larva has become differentiated in the intermediate or hypostomal sclerite. Between the anterior inner edges of the two intermediate sclerites (seen in ventral view) are two narrow sclerites which converge forwards and support the pharynx; close behind them is a cross-piece which appears to connect the intermediate sclerites. The pharyngeal sclerites have, at their anterior ends, two slender lateral processes which lie one on each side of the intermediate sclerites. The vestigial dorsal pharyngeal sclerite (*d.p.s.*) is present in the same position in the first stage larva. All the sclerites of the second stage larva are stouter, darker and more heavily chitinated than those of the first stage. The presence of the additional small tooth, the more rounded apices of the teeth, the H-piece between the intermediate sclerites and the isolation of the latter from the pharyngeal sclerites, are features which distinguish the cephalo-pharyngeal skeleton of the second stage larva from that of the first.

*Body segments.* Surrounding the margin of the head and first thoracic segment are numerous rows of minute hooks which, as in the case of the first stage larva, appear as irregular, broken rows. The rows of hooks which occur on the ventral surface at the junctions of the body segments are fewer in number than those of the preceding stage.

*Tracheal system.* The second stage larva is amphipneustic: the anterior spiracles are quite distinct and open to the exterior just in front of the posterior margin of the first thoracic segment. The stigmatic chamber expands at its anterior end and divides into five digitate processes (Fig. 5 B); at the distal end of each process is a circular area surrounded by a ring of chitin, the peritreme.

*The posterior spiracles* (Fig. 5 C) differ from those of the first stage larva in having three oval-shaped openings; these are connected through

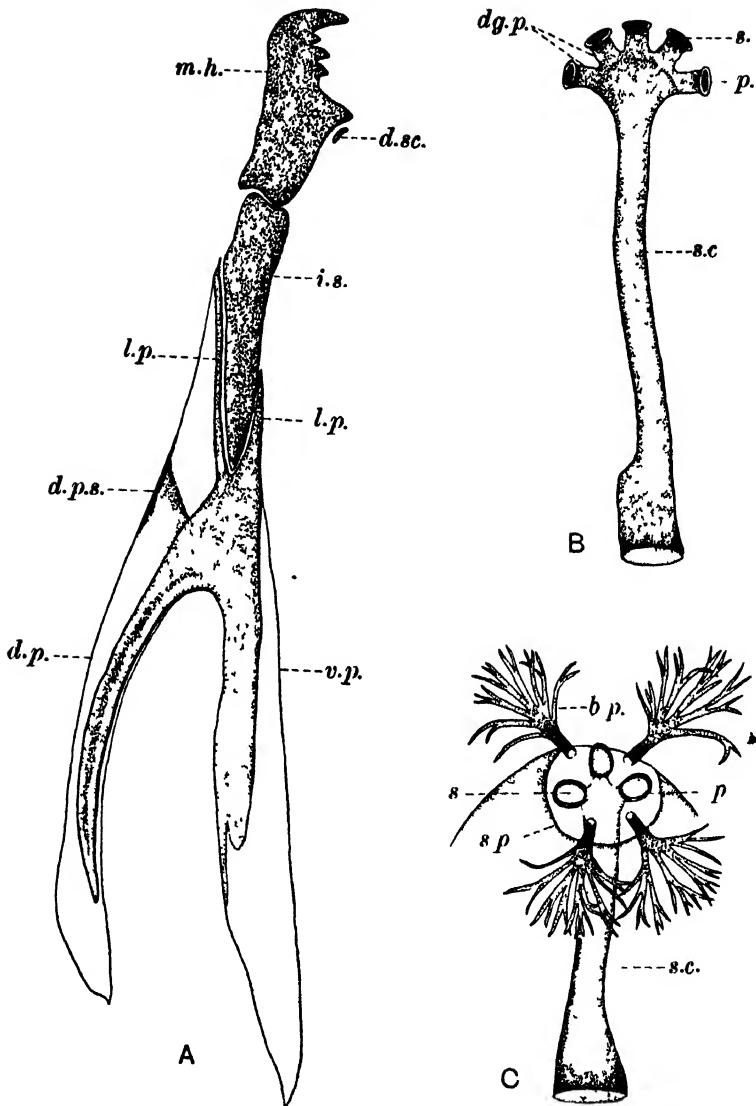


Fig 5. A. Cephalo-pharyngeal skeleton of second larval instar.  $\times 375$ . *d.sc.* dentate sclerite; *i.s.* intermediate sclerite; *l.p.* lateral process; other lettering as in Fig. 2, A.

B. Anterior spiracle of second larval instar (vertical view).  $\times 1120$ . *dg.p.* digitate processes; *p.* peritreme; *s.* spiracular opening; *s.c.* stigmatic chamber.

C. Posterior spiracle of same (surface view).  $\times 560$ . Lettering as Fig. 2 B.

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a long stigmatic chamber with the main longitudinal trunk. The four branched processes are more ramified and alternate with the spiracular openings.

The internal tracheal system of the second stage larva (Fig. 6) differs little from that of the preceding stage; the main tracheal trunks, however, extend from the anterior to the posterior spiracles. The branching is also more profuse and several paired branches run forward alongside the pharynx in the first and second segments.

### (c) *The third instar larva* (Fig. 7).

The third instar larvae vary in length from 2.8 mm. to 3.3 mm. with an average of 3.0 mm.; the maximum width ranges from 0.4 to 0.5 mm. with an average of 0.45 mm. The cuticle is firm whilst the larva has a yellowish colour due to the accumulation of reserve materials and is consequently more opaque than the first and second stage larvae. Apart from increase in size the head structures are very similar to those of the preceding larvae. The cephalo-pharyngeal skeleton (Fig. 8 A), though relatively smaller, is more heavily chitinised and shows further modification in detail. The mouth hooks are black; all the teeth have more rounded apices, and the second tooth from the anterior end is as large as the third and fourth. The intermediate and pharyngeal sclerites are heavily sclerotised; but, as in the preceding stages, they become much paler in colour towards the distal end.

*Body segments.* There are numerous rows of fine denticles around the margin, which separates the head and the first thoracic segment, but only a few rows of these structures occur along the junctions of the thoracic and abdominal segments.

*Tracheal system.* The anterior spiracles (Fig. 8 B) of the third stage larva normally terminate in six digitate processes although, in some cases, there are only five, as in the second stage larva; each process has a rounded apex, of similar structure to that of the preceding stage. The processes are, however, much longer and relatively narrower than those of the second stage larva. The posterior spiracles (Fig. 8 C) have three oval-shaped openings, each of which is connected by a separate lobe with the stigmatic chamber; the latter is continued in the main tracheal trunk (Fig. 9 B). In their general structure the posterior spiracles differ very little from those of the preceding instar. The internal tracheal system is also almost identical with that of the second stage larva.

*Remarks on the cephalo-pharyngeal skeleton* (Fig. 9 A). A transverse section, through the basal sclerite of the cephalo-pharyngeal skeleton,



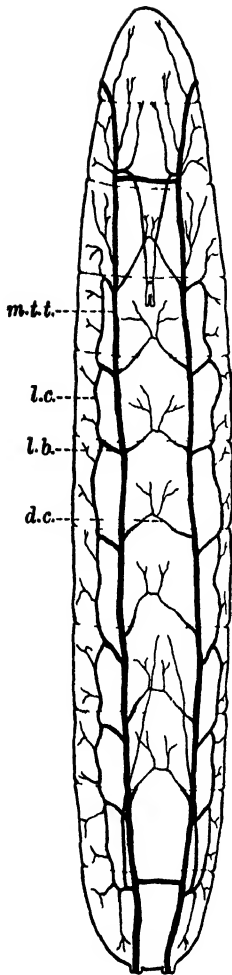


Fig. 6. Tracheal system of second larval instar  $\times 60$ . Lettering as in Fig. 3

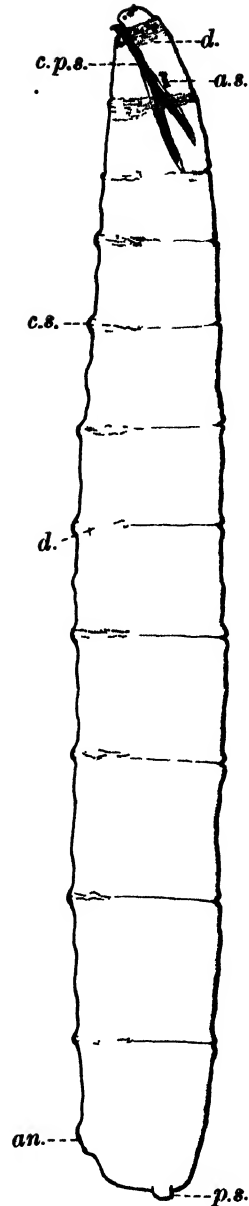


Fig. 7. Lateral view of larva in third instar.  $\times 37$ . *an.* anus; *a.s.* anterior spiracle; *c.p.s.* cephalo-pharyngeal skeleton; *c.s.* circular swellings at the junctions of the segments; *d.* denticles; *p.s.* posterior spiracle.

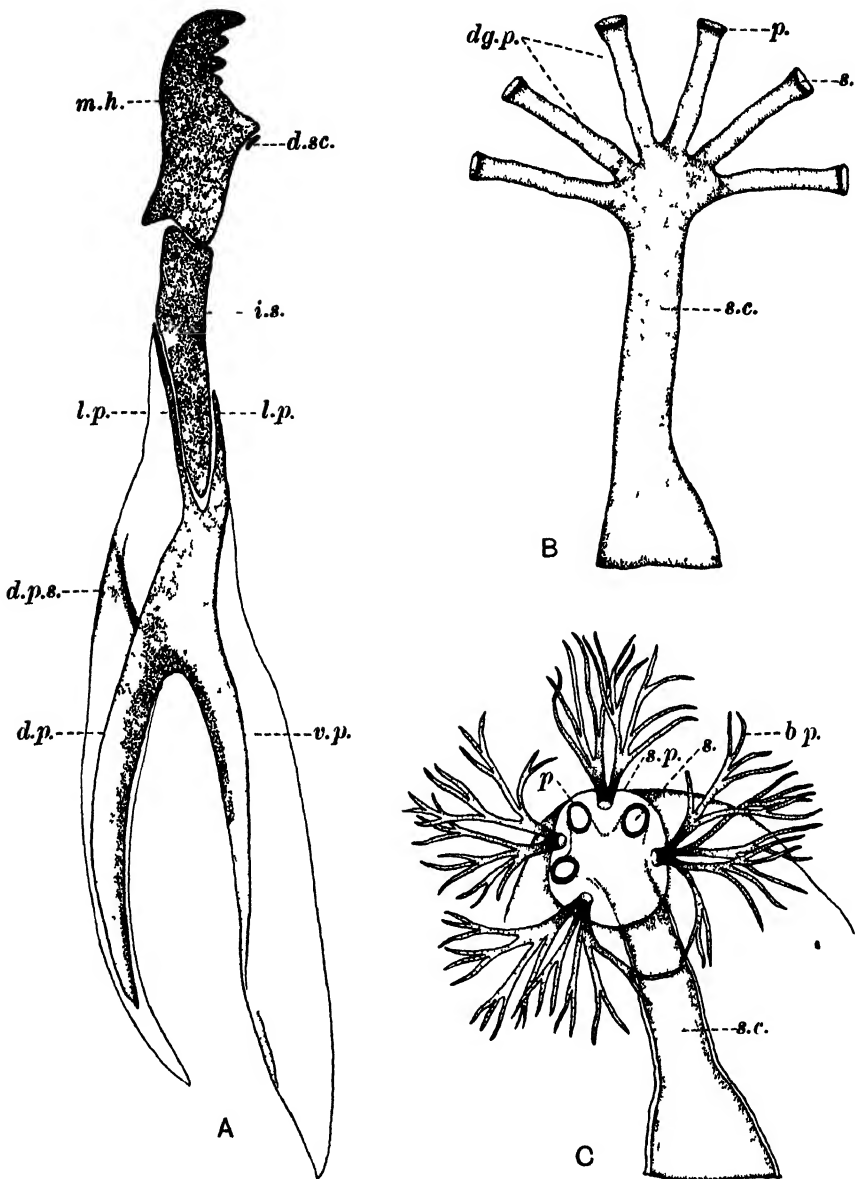


Fig. 8. A. Cephalo-pharyngeal skeleton of third larval instar.  $\times 280$  Lettering as in Fig. 5 A.  
 B. Anterior spiracle of third larval instar (vertical view).  $\times 750$ . Lettering as in Fig. 5 B.  
 C. Posterior spiracle of same (surface view)  $\times 375$ . Lettering as in Fig. 2 B.

shows a roughly oval-shaped opening, or trough, with a double ventral wall; the space between the two walls is the cavity of the pharynx. The whole trough is surrounded by a continuous cuticular rim, which is densest on the inner side, and this is also the case with regard to the cavity of the pharynx. The cavity of the trough, above the pharynx, has a lining of hypodermis which extends to the outer, upper ends of the dilator muscles. The capacity of the cavity of the pharynx is regulated by the dilator muscles which extend, from the upper wall of the trough, to the dorsal wall of the pharynx. An examination of a series of sections

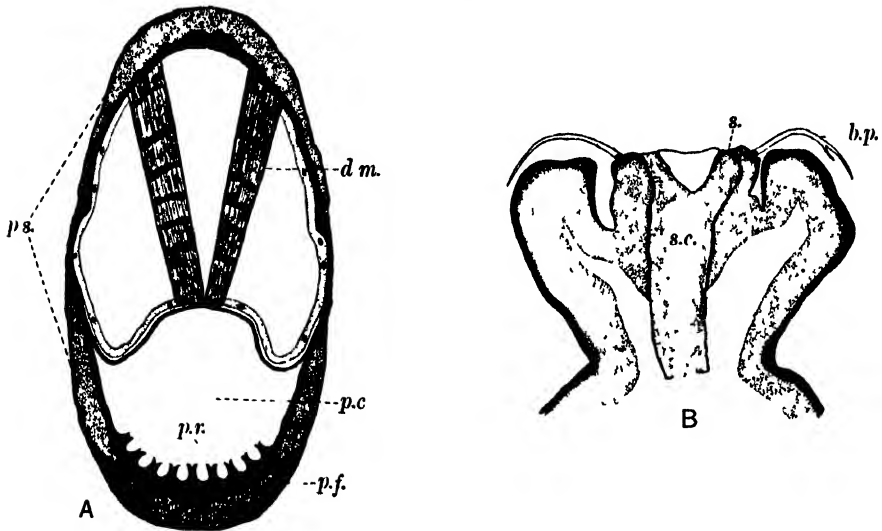


Fig. 9. A. Transverse section through pharyngeal sclerite of third larval instar.  $\times 680$ . *d.m.* dilator muscles; *p.c.* cavity of pharynx; *p.f.* floor of pharynx; *p.r.* ridges in pharynx; *p.s.* pharyngeal sclerite.

B. Longitudinal section through posterior spiracle of third larval instar.  $\times 600$ . Lettering as in Fig. 2 B.

(through the pharynx), from the anterior to the posterior end, shows a steady change in the structure of the ventral wall of the pharynx. At the anterior end the inner chitinised wall is smooth, but, as one proceeds backwards, a row of longitudinal projections, or ridges, protrude upwards into the cavity of the pharynx. These ridges, in the first few sections, are peg-like with a slight depression at their apices, but, in later sections, the depression is deeper and the ridges appear as Y-shaped structures. The lateral branches of these ridges (in some sections) almost meet, and break up the ventral surface of the pharynx into a number of small canals communicating with the rest of the pharynx by a series of slits. A few sections further back, the ridges disappear and the cavity of the

pharynx gradually decreases in diameter, especially in the dorso-ventral direction.

Keilin (4) has shown that the nature of the feeding habits of Dipterous larvae can, to a great extent, be determined by the presence or absence of these ridges in the floor of the pharynx. He maintains that all the parasitic larvae of animals, the carnivores, the predators, those which pass their whole life in the uterus of their mother (*Glossina* and *Pupipara*), and almost all the phytophagous larvae (gall-formers or miners) can be united into a vast ethnological group (larves biontophages). These larvae are deprived of ridges in their pharynx, and may be opposed to the saprophagous larvae, which have well-developed ridges. There are, however, certain phytophagous larvae, amongst which those of *Oscinella frit* have to be included, in which the ridges exist but in a form intermediate between the saprophagous and carnivorous types; the ridges are reduced and the ventral, inner wall of the pharynx is thick and heavily sclerotised. These transitional forms, Keilin is led to believe, are evolving actually under our eyes, are changing, or have changed their habitat, without their morphology having had the time to accomplish a complete cycle of transformation.

The phytophagous characters are more marked in the sclerites of the cephalo-pharyngeal skeleton described earlier in this paper. They are heavily sclerotised, the basal sclerite is deeply forked, and the dorsal and ventral processes are moderately long. The mouth hooks are toothed, whilst the surface of the head around the mouth is provided with paired dentate plates which augment the tearing capacity of the mouth hooks. A transverse section, taken near the apex of the latter, shows these dentate projections ("crochets supra-buccaux" of Keilin) at each lateral border of the mouth opening. Seen in section they resemble very closely the Y-shaped ridges which project from the ventral wall of the pharynx.

A comparison of the cephalo-pharyngeal skeleton, in the three stages, shows that this structure in the second stage bears a much closer resemblance to that of the third stage than to that of the first stage. The chief differences between the second and third stages is mainly a question of size; slight differences in form occur, but these are only in detail. There are, however, considerable differences between the cephalo-pharyngeal of the second and third stages and that of the first. The elongate accessory sclerite, which is attached to the mouth hooks in the first stage larva, seems, in the second and third stages, to have decreased in size and become fused on to the mouth hook. The dentate sclerite (Hewitt (3), Fig. 56, p. 134) which, in the first stage larva, lies between the base of

the accessory sclerite and the anterior process of the pharyngeal sclerite, appears in the second stage to have migrated forwards along the ventral edge of the mouth hook and occupies a similar position to that in the larva of *Calliphora erythrocephala* (Meijere(5); see his Figs. 157 and 158).

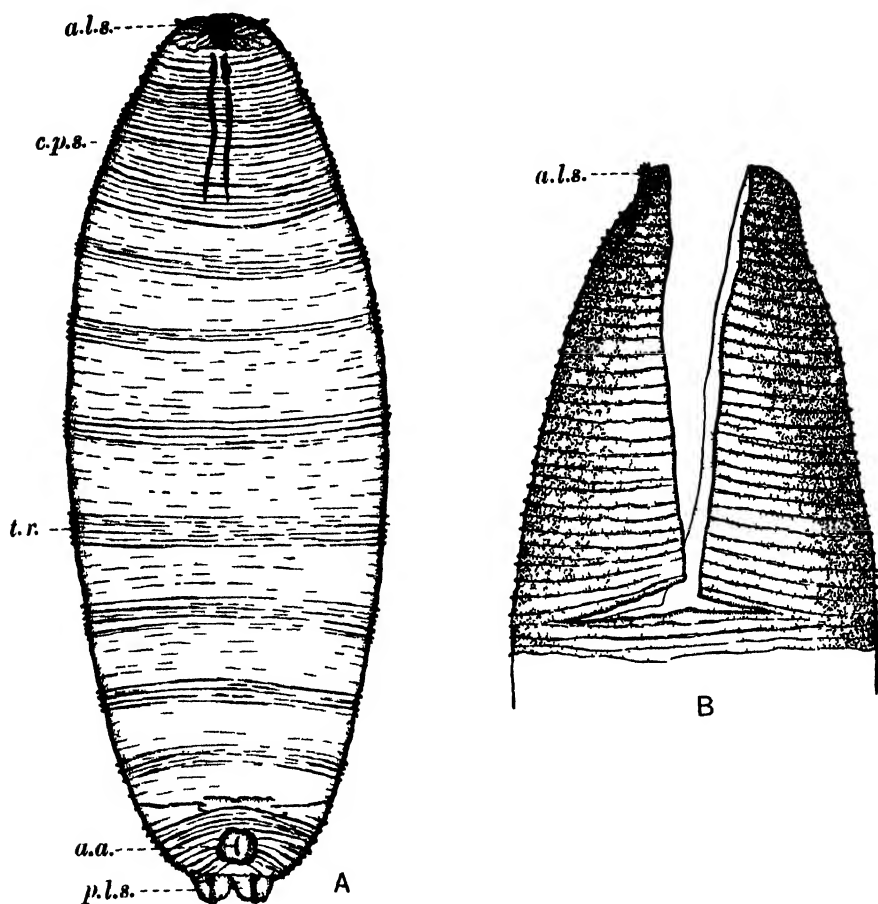


Fig 10. A. Puparium (ventral view)  $\times 37$ . *aa* anal aperture; *als* anterior spiracles; *c.p.s.* cephalo pharyngeal skeleton; *p.l.s.* posterior spiracles. *tr* transverse ridges.  
B. Puparium, showing emergence slit.  $\times 94$ .

A third important difference is seen in that the anterior process of the pharyngeal sclerite, of the first stage larva, has become separated in the second and third stages to form the H-piece or intermediate sclerite. A similar condition has been noted previously by Keilin(4) in the larval instars of *Pollenia rudis* (Keilin, Figs. 36, 37 and 38) and *Onesia sepulchralis* (Keilin, Figs. 49 and 51).

## IV. THE PUPARIUM.

The puparium (Fig. 10) is formed from the hardened integument of the third stage larva; it is reddish brown in colour, and when treated with a clearing agent the general outline of the pupal insect may be seen through it.

The puparium varies in length from 2.63 mm. to 3.08 mm. with an average of 2.8 mm.; the maximum width ranges from 0.73 mm. to 0.86 mm. with an average 0.8 mm. It is roughly barrel-shaped, tapering almost to a point at the anterior end, and is slightly flattened in a dorso-ventral direction. The integument is strengthened with transverse ridges of thickened cuticle, these are produced by the wrinkling and hardening of the integument of the third stage larva. (At the anterior and posterior poles the ridges of chitin are much thicker and harder.)

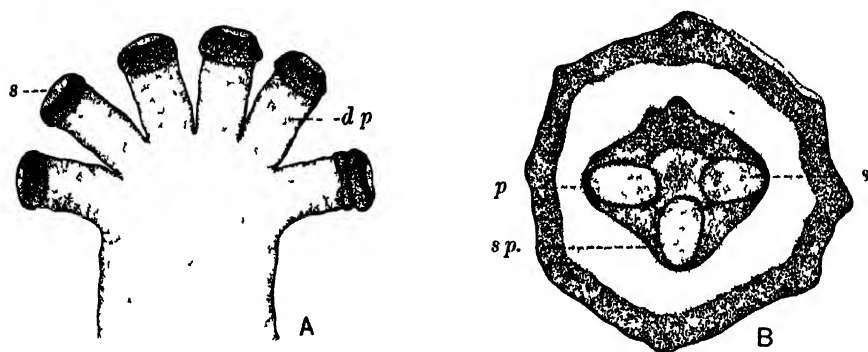


Fig 11. A Anterior spiracle of puparium (vertical view).  $\times 900$ . Lettering as in Fig 5 B.  
B Posterior spiracle of same (surface view).  $\times 900$ . Lettering as in Fig. 2 B.

The anterior spiracles (Fig. 11 A) are situated at the apex of the puparium and slightly dorsal to the median lateral line. The six digitate processes are short and darker than those of the larva, especially around the distal ends which are deep brown in colour. The basal portion of the spiracle is brownish yellow.

The posterior spiracles (Fig. 11 B) are much like those of the third stage larva. The three oval openings are situated on a dark yellowish brown spiracular plate, which is wide between the slits, but has a narrow margin at the outer edge of each. Surrounding each spiracular plate, but with an area of paler chitin in between, is a firm ring of dark brown cuticle.

*Emergence of imago from puparium.* The adult, unlike the majority of Cyclorrhaphous flies, emerges from the puparium through a horizontal,

narrow V-shaped slit (Fig. 10 B) which extends round the anterior end and backwards along each side. The pressure exerted by the imago, in emerging, causes fractures to extend from the base of the slit around the circumference of the puparium. The extent of these slits is very variable and, if pressed under a coverglass, the two portions, viz. the dorsal area bearing the two laterally situated anterior larval spiracles, and the ventral area bearing the cephalo-pharyngeal skeleton, become detached from the puparium.

#### V. SUMMARY.

1. The morphology of the immature stages of *Oscinella frit* are described and figured and certain observations of a biological nature are recorded.

2. The egg is described together with the method of eclosion of the first instar larva.

3. The structure of the larva in its three instars is described in detail with particular reference to the cephalo-pharyngeal skeleton and the spiracles.

4. The first instar larva differs markedly in structure from that in the two instars which follow. Larvae in the second and third instars are distinguishable, apart from size, only in small structural details.

5. The puparium is described, with particular reference to the spiracles, together with its method of dehiscence during the exit of the imago.

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# CALCIUM AND HYDROGEN ION CONCENTRATION AND THE INTERFACIAL TENSION OF PYRETHRUM EXTRACTS.

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(With Four Text-figures.)

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## INTRODUCTION.

THE modern theory of emulsions developed by Bancroft<sup>(1)</sup> postulates an adsorbed film round the globules; in the case of a soap being the emulsifier, the hydrocarbon chain is orientated towards the oil phase, the carboxyl group towards the aqueous phase. That alkalis have a marked effect in promoting emulsification has long been known; Donnan<sup>(4)</sup> showed that the formation of a soap was responsible, as mineral oil freed from fatty acid had the same interfacial tension in alkaline as in neutral solutions. While no one physical property can be held to account for the stability of emulsions, the interfacial tension gives generally a good indication of the probability of emulsification and, for its measurement, a drop-weight technique has been extensively used.

Hartridge and Peters<sup>(7)</sup> confirmed this method by comparison with the capillary height and a modified ripple method. Using olive oil dropped into buffer solutions, they examined the effect of the pH of

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the aqueous phase on the interfacial tension; the tension was found to fall with increasing alkalinity, becoming immeasurable at pH 9.0.

With extracts of the insecticidal plant, *Pyrethrum cinerariaefolium*, the problem of emulsification is complicated by the fact that the two poisons present, the pyrethrins, are sensitive to alkali, having been shown by Staudinger and Ruzicka (11) to be esters. An exact adjustment of the hydrogen ion concentration may, therefore, be necessary to secure the optimum conditions for the stability of both the poisons and the emulsion. Accordingly, an examination was made of the effect of the hydrogen ion concentration on the interfacial tension of pyrethrum extracts against aqueous solutions.

In addition, the influence of calcium ions was studied, as hard waters frequently have to be used in making up insecticidal washes and the soaps of divalent cations tend to promote the water-in-oil type of emulsion and even in very small amounts may cause inversion.

### METHODS AND TECHNIQUE.

*Drop-weight technique.* Measurements were made by a drop-weight method and in all the experiments the oil was run into the aqueous phase. The apparatus consisted of a 2 c.c. graduated pipette turned up at the bottom and joined to a short piece of thick-walled capillary tubing, the free end of which was ground as smooth as possible. The upper end of the pipette was fixed by means of pressure tubing to a grooved tap, the pipette filled by suction and the oil allowed to run out by its own gravity, the rate being controlled by partially closing the tap. The same pipette was used throughout and was shown to have a perfectly uniform bore. No standardisation for any error in the absolute volumes of the readings was applied, as the constant for the apparatus automatically includes this. The temperature was controlled by immersing in a beaker of water kept at 18° C. and in all the observations the drop was allowed to break away very slowly, the time per drop being between 45 and 90 seconds.

Before allowing the oil to run out, the tip was freed from oil and wetted by rubbing gently with a plug of cotton-wool, previously moistened with the aqueous solution, and forcing the oil a short way down the capillary by slight pressure. In this way it was ensured that the drop came from the inner edge of the tip. For very large drops about 1 c.c. of oil was run out. For smaller drops 70 to 150 of them were run out, an approximate value obtained by counting 5 to 10 drops and noting the volume, at intervals the number calculated to the nearest integer by taking readings as the drop broke away, and the final value obtained

by dividing the total volume by the total number of drops. When the drops were so small that there was some doubt as to the nearest whole number, 30 to 40 of them were counted directly.

*Methods of standardisation and calculation.* The apparatus was standardised by measuring the drop volume for benzene against water. The value for the interfacial tension of benzene-water was taken as 34.61 dynes/cm. interpolated for 18° C. from the accurate measurements of Harkins and Humphery(6). The constant was calculated from the following equation given by Hardy(5), from which the values for the interfacial tension,  $T_{AB}$ , were obtained:

$$T_{AB} = \frac{b(D_B - D_A)g}{rf}.$$

$b$  = volume of 1 drop in c.c.

$D_B$  = density of aqueous layer.

$D_A$  = density of oil layer.

$g$  = gravity (981).

$r$  = radius of tube.

$f$  = empirical factor.

Although this method of standardisation is open to criticism, it has been used in similar investigations and is probably valid where only comparative accuracy is required.

*Material.* The solutions were made up by diluting a highly concentrated extract of pyrethrum, prepared by treatment of the flower heads with low-boiling petroleum ether and subsequent concentration *in vacuo* at a low temperature. This extract was analysed by the rapid method of Tattersfield and Hobson(13) and found to contain 4.5 per cent. of pyrethrin I. This amount corresponds to approximately 9 per cent. of mixed pyrethrins and 45 per cent. of oleo-resinous matter, since the pyrethrins are usually present in about equal amount and constitute approximately 20 per cent. of the material extracted by petroleum ether (Tattersfield, Hobson and Gimingham(12)). A high-grade sample of semi-refined white spirit was used as the solvent.

#### RELATIONSHIP BETWEEN THE INTERFACIAL TENSION AND HYDROGEN ION CONCENTRATION.

Determinations of the interfacial tension were made against fully buffered solutions over a range of hydrogen ion concentration. The effect of the addition to the oil phase of an emulsifying agent, agrol W.B., was

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also investigated. The following solutions were made up in semi-refined white spirit and tested:

- (a) 5 per cent. pyrethrum extract.
- (b) 5 per cent. pyrethrum extract + 10 per cent. agram W.B.
- (c) 10 per cent. agram W.B.
- (d) Solvent—semi-refined white spirit.

The buffers were prepared from the “universal buffer” mixture recommended by Prideaux and Ward<sup>(9)</sup>, which consists of a solution of phenyl-acetic, phosphoric and boric acids, and yields, by the addition of varying amounts of soda, buffers over a range from pH 3.1 to 11.0. This buffer mixture possessed the advantage that it could be used throughout the experiments, but had the drawback that it was not without influence on the interfacial tension; that this effect was not serious will be shown later.

Table I. *The interfacial tension of solutions of pyrethrum extracts against buffers of varying pH.*

Oil phase	pH of buffer	Drop volume (c.c.)	Tension (dynes/cm.)	
5 per cent. of pyrethrum extract in semi-refined white spirit	3.1	0.0122	8.32	
	4.7	0.0104	7.12	
	6.0	0.00910	6.25	
	7.0	0.00815	5.62	
	8.0	0.00393	2.72	
	9.0	Immeasurable	—	
5 per cent. of pyrethrum extract and 10 per cent. of agram W.B. in semi-refined white spirit	3.1	0.00591	3.70	
	4.7	0.00544	3.42	
	6.0	0.00523	3.30	
	7.0	0.00250	1.58	
	8.0	0.00067	0.43	
	9.0	Immeasurable	—	
10 per cent. of agram W.B. in semi- refined white spirit	3.1	0.00630	3.97	
	4.7	0.00588	3.73	
	6.0	0.00549	3.50	
	7.0	0.00289	1.85	
	8.0	0.00070	0.45	
	9.0	Immeasurable	—	
Solvent: semi-refined white spirit	3.1	0.0497	34.3	
	4.7	0.0577	40.0	
	6.0	0.0634	44.1	
	7.0	0.0693	48.4	
	9.0	0.0530	37.2	
Solutions of pyrethrum extract in semi-refined white spirit at the fol- lowing concentrations:	7.0	5 %	0.00815	5.62
		10 %	0.00604	4.12
		20 %	0.00569	3.78
		50 %	0.00661	4.06
		75 %	0.00755	4.31
		100 %	0.00952	5.03

*Results.* The results are contained in Table I and represented diagrammatically in Fig. 1. The figures in Table I show that the addition of pyrethrum extract alone lowers the interfacial tension very considerably, even against acid solutions, and there must be present in the extract substances possessed of high surface activity. The extract consists of the pyrethrins, fatty and resinous material which may be conveniently described as oleo-resins, and petroleum ether. The last-named can have little effect, as its interfacial tension against water is not materially lower than that of semi-refined white spirit. Whether the

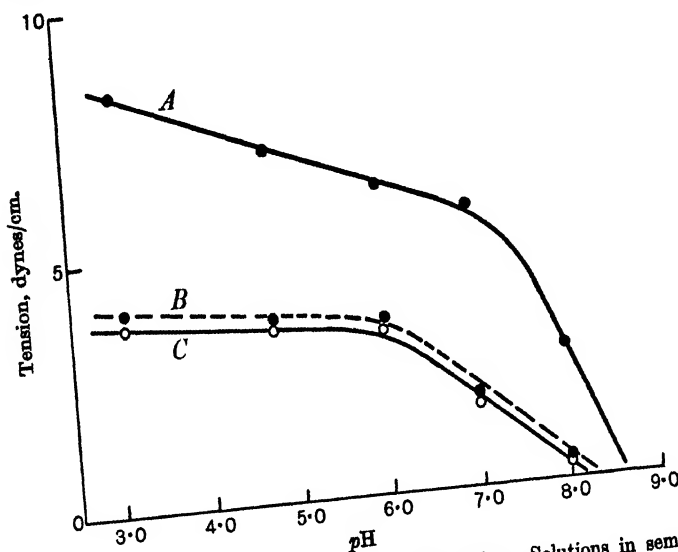


Fig. 1. pH-interfacial tension curves for oil against buffers. Solutions in semi-refined white spirit: A, 5 per cent. of pyrethrum extract; B (broken line), 5 per cent. of pyrethrum extract + 10 per cent. of agrol W.B.; C, 10 per cent. of agrol W.B.

active substances are the pyrethrins or the accompanying oleo-resins could not be determined as, unfortunately, samples of the pure pyrethrins were not available.

The effect of pH on the interfacial tension of the solution of pyrethrum extract may be seen from the curves in Fig. 1; with an increasing alkalinity the tension falls, steadily at first from pH 3.1 to 7.0, then more rapidly until at pH 9.0 stream formation occurs and the oil passes spontaneously into an emulsion. The addition of 10 per cent. agrol W.B. further lowers the interfacial tension in the extreme acid region. From pH 3.1 to 6.0 the tension of this solution drops very slightly, and from pH 6.0 to 8.0 more rapidly. The presence of the agrol W.B. appears to

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produce a lower tension and less sensitivity to the reaction of the aqueous phase than in the case of the pyrethrum extract alone; with both the tension vanishes at approximately the same *pH*. The solution of 10 per cent. agrol W.B. without pyrethrum extract possesses at all reactions a slightly higher tension than that with pyrethrum extract included, but the two curves follow each other very closely.

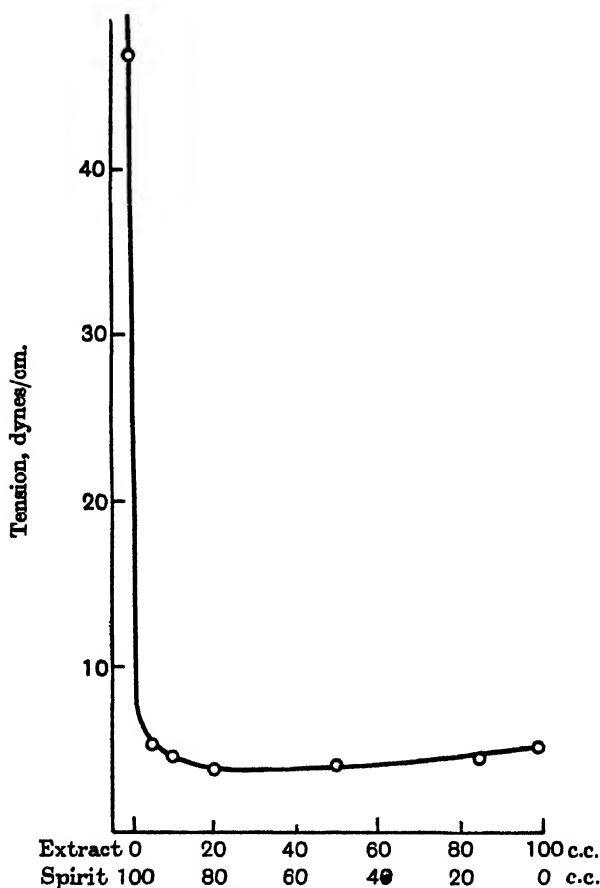


Fig. 2. Interfacial tension of mixtures of pyrethrum extract and semi-refined white spirit against buffer *pH* 7.0.

The results for semi-refined white spirit alone, given in Table I, show that the buffer mixture has probably influenced the interfacial tension, which is seen to fall with departure from neutrality on either side. Donnan (4) has shown that the tension of pure mineral oils is not affected by changing the hydrogen ion concentration. In this case the effect is possibly due to traces of fatty acid in the semi-refined white spirit, but

more probably to the presence in the buffer of phenyl-acetic acid. It is, however, not likely that the tensions of the solutions of pyrethrum and of agraal W.B. are at all disturbed, except possibly in the extreme acid region, since a weakly active substance is unlikely to exert much influence in the presence of more active ones.

#### THE EFFECT OF INCREASING THE CONCENTRATION OF PYRETHRUM EXTRACT IN THE SOLUTION.

The lowering of the interfacial tension of semi-refined white spirit by the addition of small amounts of pyrethrum extract suggested that it might be advantageous to use more concentrated solutions. The effect of the concentration of the pyrethrum extract was, therefore, studied, using a buffer of pH 7.0. The results, given in Table I and Fig. 2, show that the tension falls with increasing concentration to a minimum between 10 and 50 per cent. but the decrease is not large. It is possible that the rise in the tension with concentrations over 20 per cent. is only apparent; with increasing concentration the viscosity increases and it is well known that the drop-weight method breaks down with viscous liquids owing to the distortion of the drops, though in this case the drops had a normal appearance.

The lowering of the interfacial tension produced by increasing the concentration up to a certain limit seems hardly sufficient to compensate for the following disadvantage. As the petroleum is not without toxic action and enhances the effect of the more expensive pyrethrum, this increase of toxicity would be partly lost by using a more concentrated solution of pyrethrum extract since, for the same amount of poison in the emulsion, the less petroleum solvent would be present the more concentrated the solution.

#### THE EFFECT OF THE PRESENCE OF CALCIUM SALTS.

Whereas the soaps of monovalent cations decrease the interfacial tension between the oil and water and promote the formation of stable oil-in-water emulsions, the soaps of the divalent cations favour the water-in-oil type. Determinations were, therefore, made of the interfacial tension of solutions of pyrethrum extract against a hard water containing calcium, to which various substances were added.

For these experiments Harpenden tap-water was used; this is a typical hard water, in which emulsions are only formed with difficulty. The hardness of the water was found to be as follows: 27.6° temporary

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hardness and 30.1° total hardness as calcium; only traces of magnesium were present. Solutions were made up to contain various normalities of sodium hydroxide, carbonate and phosphate. Determinations were made of the pH colorimetrically using standards prepared from Prideaux and Ward's buffer mixture. The interfacial tension was measured with solutions in semi-refined white spirit containing 5 per cent. of the pyrethrum extract, both with and without the addition of 10 per cent. of agraal W.B.

The aqueous solutions were not fully buffered, but it appears unlikely that the pH at the interface would be seriously disturbed as neither the pyrethrum extract nor the agraal W.B. are strongly acid, and the concentration of each was low. Hartridge and Peters(7), working with pure oleic acid dropped into *N*/100 soda, found that the drops were small if formed rapidly and large if formed slowly; this abnormality they explained as the result of neutralisation when the drop formed slowly. Changing the rate of drop formation did not have this effect on the drop volume in my experiments, and there can be little doubt that no significant changes in the pH occurred at the interface.

The results with Harpenden tap-water are given in Table II. In Fig. 3 the values for the interfacial tension are plotted against the hydrogen ion concentration, the curves obtained with the buffers (free from calcium) being repeated for purposes of comparison.

Table II. *The interfacial tension of solutions of pyrethrum extract in semi-refined white spirit against Harpenden tap-water containing added solutes.*

Aqueous phase		Tension: dynes per cm.	
Solute	pH	5 % pyrethrum extract	5 % pyrethrum extract + 10 % agraal W.B.
— (tap-water)	6.9	7.18	4.76
0.04 <i>N</i> NaCl	6.9	—	5.00
0.01 <i>N</i> Na <sub>2</sub> CO <sub>3</sub>	8.4	5.59	2.79
0.02 <i>N</i> „	9.5	Immeasurable	Immeasurable
0.04 <i>N</i> Na <sub>2</sub> HPO <sub>4</sub>	7.6	5.20	2.76
0.12 <i>N</i> „	8.3	3.02	0.6
0.001 <i>N</i> NaOH	8.5	7.3	4.8
0.005 <i>N</i> „	9.8	6.7	4.5
0.007 <i>N</i> „	10.1	5.4	1.6
0.010 <i>N</i> „	10.9	Immeasurable	Immeasurable
0.5 % agraal I	—	0.3	—

The effect of a small amount of calcium salt is well illustrated by the result obtained with the solution of pyrethrum extract and agraal W.B. against untreated tap-water in which the concentration of calcium was 0.003 moles per litre (corresponding to *p*Ca 2.5) and the pH 6.9. The interfacial tension was found to be 4.76 dynes, the value for the buffer



solution of this pH being 1.8 dynes (interpolated). With this solution the interfacial tension appears to be more sensitive to the presence of calcium ions than of hydrogen ions, since the tension against the most acid buffer tested (pH 3.1) was 3.7 dynes and from the flatness of the curve in this region would probably be under 4.0 dynes at pH 2.5; with tap-water of  $pCa$  2.5 the value was 4.76 dynes. When the agram W.B. is absent, hydrogen ion is the more effective as the tension with the buffer at pH 3.1 (8.32 dynes) is higher than with tap-water of  $pCa$  2.9 (7.18 dynes).

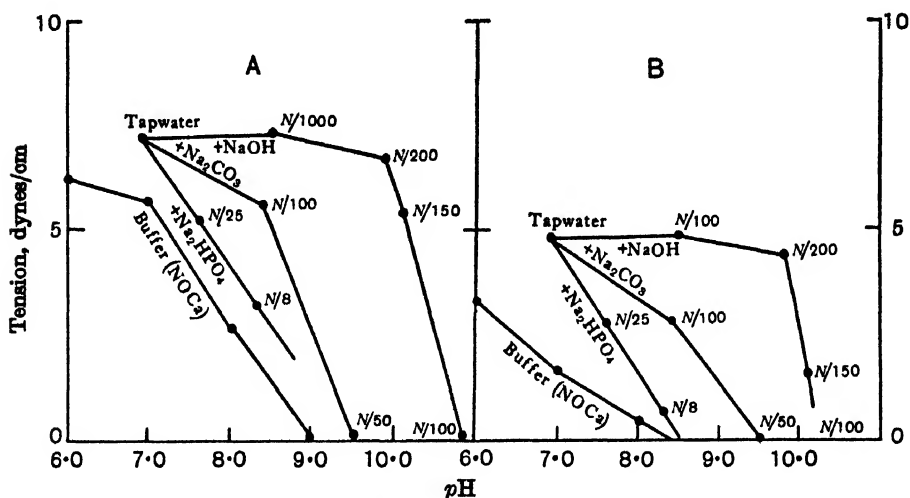


Fig. 3. pH-interfacial tension curves for oil against tap-water containing calcium and added salts and against calcium-free buffers. A, 5 per cent. solution of pyrethrum extract in semi-refined white spirit; B, 5 per cent. solution of pyrethrum extract in semi-refined white spirit + 10 per cent. agram W.B.

The results obtained with tap-water containing a wetter, agram I, raises an interesting point. It will be seen from Table II that the addition of agram I has decreased the tension of the pyrethrum extract solution against tap-water from 7.18 to 0.3 dynes. This effect is probably due to the lowering of the surface tension of the water and adsorption of the agram I at the interface. When mixtures of pyrethrum extract solutions and water containing agram I were gently shaken, they passed readily into an apparent emulsion, which broke just as readily in one or two minutes. Evidently agram I lowers the tension without stabilising the emulsion.

An examination of Fig. 3 shows that increasing the alkalinity by adding sodium hydroxide to the tap-water lowers the interfacial tension; the results with sodium carbonate and phosphate solutions indicate that

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the tension can be reduced at lower hydroxyl ion concentrations by precipitating the calcium. In order to study more closely the relationship between the interfacial tension and the concentration of calcium and hydroxyl ions in the aqueous solution, the amounts of calcium present in the solutions used were calculated.

The methods of calculation and the data employed were those given by Hastings, Murray and Sendroy<sup>(8)</sup> and Sendroy and Hastings<sup>(10)</sup> in their studies on the solubilities of calcium carbonate and calcium phosphates in biological fluids. The values for the concentrations of carbonate ions were calculated from the amounts of carbonate known to be present without reference to the atmospheric CO<sub>2</sub> tension, since the solutions were not brought into equilibrium with the air and could absorb but little carbon dioxide during the experiments. In the solutions containing sodium hydroxide, which gave little or no precipitate unless heated and were evidently supersaturated with calcium carbonate, the original amount of calcium was assumed to be present. No precautions were taken to prevent supersaturation in any of the solutions; however, since the sodium carbonate and phosphate solutions were prepared by diluting strong solutions in tap-water, the solid phase was present at the moment of preparation. It has also been tacitly assumed that the presence of the precipitate was without influence.

Table III. *The interfacial tension of solutions of pyrethrum extract in semi-refined white spirit and the ratios  $\frac{[\text{Na}^+]}{[\text{Ca}^{++}]}$  and  $\frac{[\text{OH}']}{[\text{Ca}^{++}]}$  in the aqueous phase.*

Tap-water solution						Tension: dynes per cm.	
Solute	Nor- mality	<i>a</i> <i>p</i> Ca <sup>++</sup>	<i>b</i> <i>p</i> O'H	$\log \frac{[\text{Na}^+]}{[\text{Ca}^{++}]}$	$\log \frac{[\text{OH}']}{[\text{Ca}^{++}]}$	5 % pyre- thrum extract	5 % pyre- thrum extract + 10 % agral W.B.
Na <sub>2</sub> CO <sub>3</sub>	0.01	4.4	5.7	2.4	-1.3	5.6	2.8
"	0.02	5.4	4.6	3.7	+0.8	Immeasurable	Immeasurable
Na <sub>2</sub> HPO <sub>4</sub>	0.04	5.1	6.5	3.5	-1.4	5.2	2.8
"	0.12	5.6	5.8	4.8	-0.2	3.0	0.6
NaOH	0.001	12.5	5.6	-0.5	-3.1	7.3	4.8
"	0.005	12.5	4.3	0.2	-1.8	6.7	4.5
"	0.007	12.5	4.0	0.3	-1.5	5.4	1.6
"	0.01	12.5	3.2	0.5	-0.7	Immeasurable	Immeasurable
—	—	2.5	7.2	?	-4.7	7.2	4.8

Table III gives the concentrations of sodium, calcium and hydroxyl ions, the logarithms of the ratios of sodium and of hydroxyl to calcium ion concentrations, and the interfacial tensions. Evidently, neither the concentration of calcium or hydroxyl ion nor the ratio of sodium to

calcium ions bears any relation to the interfacial tension figures; on the other hand, the values for the logarithm of the ratio of hydroxyl to calcium ion concentration appear to show a definite correlation. This is illustrated in Fig. 4, in which the interfacial tension is plotted against

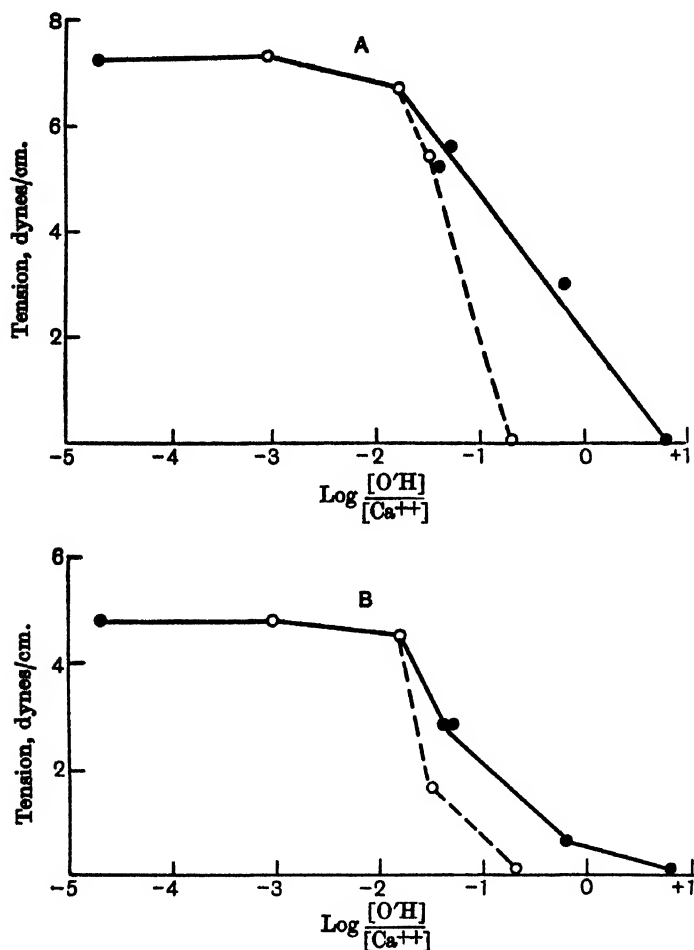


Fig. 4. Interfacial tension and the logarithm of the ratio of calcium to hydroxyl ions in the aqueous phase. Oil phase: A, 5 per cent. solution of pyrethrum extract in semi-refined white spirit; B, 5 per cent. solution of pyrethrum extract in semi-refined white spirit + 10 per cent. agrol W.B. Aqueous phase: tap-water with added NaOH,  $Na_2CO_3$ ,  $Na_2HPO_4$ . Results with NaOH ringed and, where they diverge, shown by broken line.

$\log \frac{[O'H]}{[Ca^{++}]}$ ; with the exception of the values for the sodium hydroxide solutions which are treated separately, the tension falls steadily as the logarithm of the ratio increases from -1 to +1 at which point the

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tension vanishes. The curves obtained are not dissimilar to those (Fig. 1) in which the interfacial tension is plotted against the  $pH$  in the absence of calcium.

In the case of the sodium hydroxide solutions, as the figures for  $pCa$  are uncertain and supersaturation has been shown to be present, it is not surprising that the results are analogous. Nevertheless, it is possible that at the higher concentrations the critical ratio may not be the same. The effect of calcium ions is presumably due to their adsorption, which would be relatively less at high concentrations, and the ratio of hydroxyl ion to calcium ion adsorbed at the interface, rather than the ratio in the solution, is probably the determining factor. An alternative possibility is that, at the higher alkalinities, changes such as hydrolysis may occur in the oil at the interface.

### DISCUSSION.

It has been shown that the addition of a pyrethrum extract lowers the interfacial tension of semi-refined white spirit against aqueous solutions to a marked degree (Table I). Whether this result is due to the pure poisons, the pyrethrins, or to the concomitant oleo-resinous matter, has not been determined, but it seems likely that the resins are mainly responsible as they are known to possess good emulsifying properties.

With increasing alkalinity in the aqueous phase the tension decreases (Fig. 1). Hartridge and Peters (7), working with olive oil, found that a rapid fall in the tension occurred at  $pH$  5.0 and related this to the change in volume of the carboxyl group, which occurs at this  $pH$ . With pyrethrum extracts the tension falls gradually from  $pH$  3.0 to  $pH$  7.0 and then more rapidly; that the whole change is spread over a wider range of  $pH$  and is less abrupt than with olive oil, may be explained by supposing that an acid group of a different nature is operative. The results obtained when agral W.B. is added (Fig. 1) confirm this view; thus, the introduction of a different acid group completely alters the relationship between the interfacial tension and the reaction of the aqueous phase. Further, the more active agral W.B. seems to supersede the effective constituent in the pyrethrum extract, as the curves for solutions of the two together and of the agral W.B. alone are almost identical.

The presence of calcium salts in the aqueous phase raises the tension of pyrethrum solutions (Fig. 3), and the addition of agral W.B. to the oil decreases the effect of calcium ions as it does that of hydrogen ions. Alkaline salts counteract the influence of calcium salts and the order of

efficiency at the same pH has been found to be sodium phosphate, sodium carbonate and sodium hydroxide. This is also the decreasing order both of the valency of their anions and of their power to precipitate calcium salts.

An examination of the concentrations of different ions in these solutions has shown that the ratio of hydroxyl to calcium ions can be correlated with the interfacial tension values. It is of interest to note that both Clowes<sup>(3)</sup> and Bhatnagar<sup>(2)</sup> came to similar conclusions, using different oil emulsions and relatively high concentrations of divalent salts. Clowes showed that sodium salts counteracted the effect of calcium salts on olive-oil emulsions, the critical ratio being 100 mols of sodium chloride, or 4 mols of sodium hydroxide, to 1 mol of calcium chloride. Bhatnagar found a critical ratio of 4 mols of potassium hydroxide to 1 mol of barium nitrate with paraffin-oil emulsions. For the emulsions of pyrethrum extract (Table III) the critical ratio of hydroxyl to calcium ion appears to lie between 1 and 10 when the calcium concentration is low. Clowes attributed the effect of sodium salts to the adsorption of the anion, the cation playing no part. My experiments confirm this view since the ratio of sodium to calcium ion concentration appears to have no effect on the tension (Table III), even when it rises to over 1000.

It is not certain that softening agents improve oil emulsions in hard waters only by lowering the interfacial tension. This value measures the resistance to mixing of two immiscible liquids and, when it approximates to zero, the two liquids will readily pass on shaking into an emulsion which may or may not be stable. The interfacial tension, therefore, cannot be regarded as a reliable index of the stability of the resulting emulsion. In the case of the insecticidal emulsions it is essential to have information as to the breaking properties of the emulsion; an account of direct investigations on this question will be included in a separate publication.

The author is indebted to Dr F. Tattersfield for suggesting this investigation and its close bearing on the emulsification of pyrethrum extracts and for his active interest in the work and the writing of this paper.

#### SUMMARY.

1. The addition of a pyrethrum extract to a petroleum solvent, semi-refined white spirit, considerably lowers its interfacial tension against water. The tension also depends upon the reaction of the aqueous phase, decreasing as the alkalinity increases.

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2. The addition of agram W.B. to a solution of pyrethrum extract further lowers the interfacial tension more especially against acid solutions, thereby decreasing the sensitivity of the tension value to the pH of the aqueous phase.

3. The presence of calcium salts in the aqueous phase raises the interfacial tension of solutions of pyrethrum extract.

4. Alkaline salts counteract the effect of calcium salts, and the resulting tension values can be correlated with the ratio of calcium to hydroxyl ion concentration.

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# THE EVALUATION OF PYRETHRUM FLOWERS (*CHRYSANTHEMUM CINERARIAEFOLIUM*).

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(With Plate I and Three Text-figures.)

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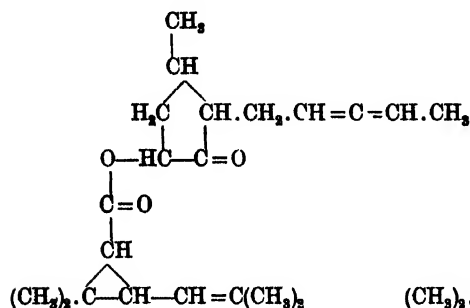
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## INTRODUCTORY.

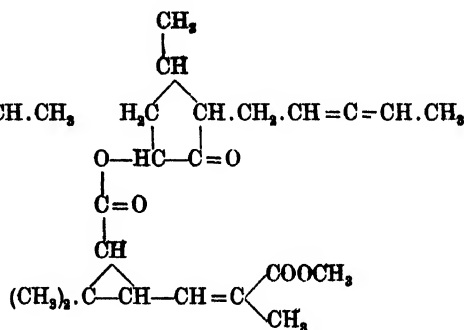
THE extended use of pyrethrum (*Chrysanthemum cinerariaefolium*) as an insecticide in recent years and the necessity of increasing the production of this plant have demanded better methods of evaluation of the active principles. This demand will be accentuated if, as seems probable, an intensive campaign of selection for higher content of the pyrethrins is undertaken. The work of Staudinger and Ruzicka (4) on the constitution of the pyrethrins has already led to suggestions of methods of chemical analysis associated with the names of Staudinger and Harder (5), Tattersfield, Hobson and Gimingham (6) and of Gnadinger and Corl (1). Although further work is still needed to ascertain whether one or all of these methods are suitable for detecting the loss of toxicity associated with exposure of the finely powdered flowers and certain extracts to the

atmosphere, the methods proposed may be regarded as satisfactory for the recently harvested crop or carefully stored flowers. None are fool-proof, and there would appear to be conditions under which relatively inaccurate results may be obtained. We propose in this paper to examine some of the difficulties associated with certain of these methods. In addition, it is desirable to have methods applicable to the produce of a single plant and, if possible, for the evaluation of a single flower head—we therefore also suggest two new methods which, although perhaps not susceptible to the same degree of accuracy as the older methods, require much less material and are likely to be of use for this purpose.

The active principles, namely pyrethrins I and II, are associated in the plant with other organic compounds, and are esters resulting from the condensation of a ketonic alcohol (pyrethrolone) and two acids, one monobasic, volatile in steam, and the other dibasic and soluble in water.



Pyrethrin I



Pyrethrin II

It is not outside the range of possibility that in the flowers small amounts of the pyrethrolone may exist associated with other acids than the two pyrethrin acids, and that the latter may in small amount be condensed with some other alcohol. It is, therefore, of some importance to ascertain the degree of concordance between the results obtained in the determination of the active principles by means of the estimation of the acidic portion and those by the determination of the ketonic-alcohol fraction of the pyrethrin molecules. Tattersfield, Hobson and Gimingham(6) reduced the two methods originally suggested by Staudinger and Harder(5) to a microscale and compared them; relatively close agreements were obtained between the acid method and the semicarbazone method, but it was felt that the latter was too complicated and tedious for general use. More recently Gnadinger and Corl have published a much more rapid method of ascertaining the active principles depending upon their reducing properties, which are associated with the

pyrethrolone portion of the pyrethrin molecules. We have compared the results obtained by these methods, and although for samples of high pyrethrin content certain modifications will have to be suggested, so far results have been obtained which are in comparatively close agreement. Concordance of a very high order is hardly to be expected, however desirable, but it must also be recognised that small differences in pyrethrin content can only be detected, if at all, with great difficulty by means of the biological insecticide tests at present available. Moreover, owing to wide variations in insect resistance to the toxic effects of pyrethrum, even with the same species, an ample margin of safety should always be allowed in spraying practice, and this tends partially to off-set the higher value of a sample with only a slightly greater poison content than another. The agreements obtained are, in our opinion, sufficiently good therefore to warrant their use for evaluating samples, for the standardisation of extracts, and to standardise the newer methods here suggested.

In carrying out a quantitative investigation on the change in amount of the active principles during the development of the flowers from the small bud stage to the fully open flowers, there was found to be a considerable variation from plant to plant grown from selected seed from the same source, and with the flowers at the same stage of development. The analysis of a fairly large number of plants had to be undertaken in order, as far as possible, to allow for these variations, and the occurrence of one or two plants of an exceptional nature might easily have led to erroneous deductions as to the stage at which the average pyrethrin content was at a maximum. It was thus decided to search for a method requiring so small an amount of material that the whole, or at any rate a large part, of such an investigation could be carried out on the flowers derived from one plant.

It was found that the flowers of certain plants were very rich indeed in pyrethrin content and that for such samples the acid method as outlined by Tattersfield, Hobson and Gimingham<sup>(6)</sup> tended to give low results. Some modification was obviously needed. The modification suggested is, in the main, that the weight taken for extraction should be reduced for samples containing more than a certain percentage of the total pyrethrins, and that greater care be taken to secure their complete hydrolysis. An alternative method which could be used for rapidly ascertaining, if only approximately, this percentage would be of value, which would be all the greater if only a small amount of the sample were required. If necessary the acid method could then be carried out to determine the actual amount of the two separate constituents.

## EXPERIMENTAL.

*The acid method.*

In the course of an investigation by one of us of the flower heads from separate plants, a degree of variation in percentage pyrethrin content was observed. In a number of cases this value was higher than we had hitherto observed or had thought was to be expected when the original acid method was devised. It was decided to check these values and, in order to assure complete hydrolysis of the esters, to use a smaller weight of flower heads and a greater relative amount of alkali for the hydrolysis. The results, particularly for pyrethrin II, frequently came out higher than when the method as originally described was employed.

In Table I are recorded data indicating the kind of discrepancy that may occur.

Table I.

Sample no.	Pyrethrin I (%)	Pyrethrin II (%)	Total pyrethrins (%)	Weight used (gm.)
G. 1	0.69	0.65	1.34	10.0
	0.68	0.73	1.41	5.0
	0.68	1.02	1.70	2.5
H. 10	0.50	0.71	1.21	10.0
	0.52	0.92	1.44	5.0
	0.50	1.00	1.50	2.5
G. 4	0.96	0.72	1.68	5.0
	0.95	0.94	1.89	2.5
G. 9	0.61	0.82	1.43	10.0
	0.63	1.06	1.69	5.0
	0.63	1.14	1.77	2.5

For these high samples where 10 gm. has been employed, the values are on the low side, and although this may be due to imperfect hydrolysis or incomplete extraction of the dicarboxylic acid or to both, adsorption during filtration of the dicarboxylic acid after the distillation of the volatile acid appears also to be involved. In a previous investigation (6) it was noted that a small quantity of animal charcoal will adsorb practically the whole of the dicarboxylic acid and when, as in such samples as these, the acid is fairly highly concentrated in the residue after distillation, it may be partially held back on the cotton-wool pad. On the other hand, where 2.5 gm. is used, very careful titration indeed is required, as the smaller weight used leads to a proportionally greater experimental error. We have adopted in more recent work the following modifications of the original method. For samples of poor quality—below 0.7 per cent. total pyrethrins we have extracted with petroleum ether 10 gm., for

samples between 0.7 and 1.5 per cent., 5 gm. and for samples above 1.5 per cent., 2.5 gm. After taking down to a small bulk in a current of  $\text{CO}_2$  and evaporating the residue in a vacuum desiccator, the residue is extracted with four lots of 2.5 c.c. each of gently warmed purified methyl alcohol, each of which is cooled and filtered into a 100 c.c. Kjeldahl flask through a pad of cotton-wool. A final washing with 2.5 c.c. cold methyl alcohol is made, a few drops of phenolphthalein in methyl alcohol are added and then, drop by drop till just alkaline, a solution of caustic potash in methyl alcohol of  $N/1$  strength. A further 5 c.c. are added and the mixture refluxed for a full 8 hours. The methyl alcohol is taken off in partial vacuum with gentle warming (the temperature not being allowed to rise above  $25^\circ \text{C}.$ ), the residue dissolved in water and the volatile acid distilled off in steam. The volume in the distillation flask should not be allowed to exceed 30 c.c. Two lots of 50 c.c. are distilled off and the acids in the first distillate extracted with two lots of 50 c.c. of petroleum ether, each extract being washed with 20 c.c. of distilled water. The two extracts are combined, evaporated on a water bath after addition of 20 c.c. of distilled water, and the residue titrated while warm with  $N/50$  soda, the sides of the flasks being washed down towards the end with a little neutral methyl alcohol. From the titration the amount of pyrethrin I can be determined (6). The second distillate of 50 c.c. may be extracted with petroleum ether; it should not show more than a trace of titratable acid. The hot aqueous residue in the distillation flask is treated with 0.2 gm. of calcium sulphate and, after standing overnight, filtered through a cotton-wool plug, washed three or four times with water and extracted exhaustively with sodium-treated ether in the apparatus already described (6). In a rapid extractor 20 hours' extraction appears to be the minimum time necessary for complete extraction of the dicarboxylic acid in the case of samples of high pyrethrin content. After adding 20 c.c. of distilled water the ether is evaporated, the aqueous layer heated to boiling, cooled and filtered through a cotton-wool plug and the filtrate, after heating to boiling, titrated with  $N/50$  soda.

1 c.c.  $N/50$  alkali = 3.36 mg. monocarboxylic acid = 6.6 mg. Pyrethrin I  
= 1.98 mg. dicarboxylic acid = 3.74 mg. Pyrethrin II.

The apparatus employed is precisely the same as that described by Tattersfield, Hobson and Gimingham (6). The method requires considerable care if accurate results are to be obtained and, although the determination of pyrethrin I has not presented any great difficulty, the estimation of pyrethrin II is not free from technical difficulties and makes the process rather prolonged. We describe below a short method of

determining the total pyrethrins, which for flowers in the later stages of development, i.e. later than the small bud stage, has given results for the total pyrethrins in general concordance with both the acid and the Gnadinger and Corl methods. It is probable that by its use, combined with the present method of determining pyrethrin I, a sufficiently accurate value for pyrethrin II would be obtained in a shorter time and with less technical difficulty. This simplification is now under investigation.

*The acid method and Gnadinger and Corl method compared.*

We have tested a number of samples by both these methods, the results being given in Table II.

Table II. *Comparison of results by acid method and method of Gnadinger and Corl.*

Sample no.	Acid method		Total pyrethrins (%)	
	Pyrethrin I (%)	Pyrethrin II (%)	Acid method	Gnadinger-Corl
B.	0.21	0.42	0.63	0.65
E.M. 2	0.45	0.60	1.05	1.06
E.M. 3	0.51	0.44	0.95	1.03
E.M. 6	0.40	0.58	0.98	0.94
E.M. 7	0.36	0.87	1.23	1.16
D.L.†	0.24	0.32	0.56	0.73
F. 11†	1.32	0.86	2.18	2.30
Sw.†	1.13	1.02	2.15	1.98
42*† (1)	0.20	0.24	0.44	0.40
(2)	0.20	0.22	0.42	—
82* (1)	0.38	0.60	0.98	0.92
(2)	0.39	0.60	0.99	—
G. 5 (1)	0.77	0.76	1.53	1.49
(2)	0.77	0.73	1.50	—
(3)	0.74	0.73	1.47	—
L.M.† (1)	0.67	0.87	1.54	1.51
(2)	0.74	0.84	1.58	1.47
(3)	0.66	0.99	1.65	—

\* These samples were received from Messrs Gnadinger and Corl, who permit us to give their values: Sample 42, 0.39, 0.39; sample 82, 0.97, 0.97, 0.97, 0.94, in terms of total pyrethrins per cent.

† The ferriocyanide Method A, to be described later (p. 125), gave results as follows: D.L. 0.70, F. 11, 1.63, Sw. 2.05, No. 42, 0.40, and L.M. 1.58, in terms of total pyrethrins per cent.

These samples, with the exception of Nos. 42, 82, and D.L., were all grown upon experimental plots from selected seed. They were known to be genuine *Chrysanthemum cinerariaefolium*, and were harvested and dried with the greatest care. For purposes of comparison, there are given also a few of the values obtained by the ferriocyanide method, to be described later (p. 125). Four of the samples, 42, 82, L.M. and Sw., were



also tested by a biological method, in order to ascertain if there were concordance between the analytical data and the insecticidal values (p. 122). With one exception (D.L.) there cannot be said to be any very serious discrepancies between the results obtained by the different methods; the differences shown would scarcely be susceptible of detection by biological means. The sample D.L., in which the largest percentage discrepancy occurs between the results obtained by the acid method and the two others, was a commercial sample and seemed to us exceptional. It possessed an odour different from that of genuine pyrethrum.

In using the Gnadinger and Corl method we have introduced one minor modification, in that we have put the dextrose standard through exactly the same processes as the pyrethrum samples, except that of extraction with petroleum ether; thus the presence of any reducing matter in the alcohol used, although very small in any case, has not called for any correction. As thus applied, the Gnadinger and Corl method has proved of considerable value to us in checking samples of flowers from our experimental plots, which gave results which were unexpectedly high. In genuine samples, in the analysis of which great care has been exercised, we have so far had no serious discrepancy over a range of pyrethrin content of 0.40 per cent. to more than 2 per cent. Our experience of commercial samples has not been sufficiently great for us to say how great a concordance could be obtained in their case. Adulteration or fermentation due to inadequate drying or bad storage would possibly lead to discrepant results.

#### *Errors due to "shaking-out" of sample.*

During the course of the above investigations, we have noticed changes in the pyrethrin content of individual samples after being kept in tightly corked tubes for some months. This we have traced to a gradual "shaking-out" of the samples. In order to avoid formation of a hard cake during the petroleum ether extraction, with subsequent incomplete exhaustion, the samples were not ground too finely, approximately only 26 per cent. passing through a 100-mesh sieve. It was noticed that this finer fraction tended to separate out towards the top of the tube as the sample was kept, and was, moreover, very high in pyrethrin content. The fine fractions of the sample apparently consist of the more brittle parts of the flowers, namely the achenes, which, as Gnadinger and Corl<sup>(2)</sup> have shown, contain approximately 90 per cent. of the total pyrethrins of the flower heads. The fine powder obtained by

passing a normally ground sample through a 100-mesh sieve was found to contain 3.3 per cent. of total pyrethrins when tested by reduction of ferricyanide (Method A, p. 125), the residue on the sieve having a content of 1.30 per cent. The pyrethrin content calculated from the weights of the fractions and the determined content of each, agreed exactly with the figure obtained by analysis of the well-mixed samples (1.85 per cent.). It will be seen that a slightly irregular distribution of the more finely ground fractions of samples, such as we have been using, may lead to widely differing figures upon analysis. In order to avoid the possibility of erroneous results due to this factor, we suggest the samples be ground to an impalpable powder, and mixed with ignited sand prior to extraction with petroleum ether.

#### INSECTICIDAL TESTS.

The direct correlation of the analytical results with the insecticidal properties of samples showing widely ranging pyrethrin contents was realised as being of importance. We chose two commercial samples sent to us from the United States (Nos. 42 and 82), and two samples grown upon half-acre experimental plots and harvested in July 1930 and labelled L.M. and Sw. The analytical results are given in Table III, the samples showing total pyrethrin contents of 0.40 to 2 per cent. Each was extracted with absolute alcohol in the cold, the 10 per cent. extract was then diluted with saponin solution of 0.5 per cent. concentration, and the dilutions shown in Table III used for spraying.

The insects used were *Aphis rumicis*, and the trials were carried out in the way and with the apparatus already described(7). Random samples were not taken of the whole of the insects available, but the adult apterous females were selected with considerable care and, for every concentration tested, ten insects were used. Repetitions were carried out on the same day at certain of the concentrations. After spraying, the insects, without further handling, were placed in petri dishes near fresh bean foliage and examined each day for 3 days. Each test was given a number, and the examination carried out and the result expressed in ignorance of the actual concentration to which it referred. The insects were put into four categories: (1) those not affected, N; (2) those somewhat affected but able to walk, S; (3) the moribund, i.e. those able to move appendages, M; (4) those apparently dead, D. Each individual insect was carefully examined each day to ascertain the extent of the toxic effect produced. The observations were not carried out later than

the third day after spraying, as the insects in the controls were showing signs of failure on that day; these few failures appear to have been generally due to the insects involved not having settled down on the foliage provided. The tests were carried out with concentrations ranging from those giving 100 per cent. moribund and dead insects to those in which the mortality was nil. The data should be inspected as a whole but, for purpose of ready comparison, we have given the percentage value of the moribund and dead taken together, and in addition have awarded marks by the addition of the percentage number of dead to half the percentage of moribund and one-quarter of the percentage of the slightly affected. By this latter method the grading effect is more definitely indicated by a single figure than by the former, but the toxic action is probably under-valued.

Table III. *Toxicities of pyrethrum samples to Aphis rumicis.*

		Marks=[S/4 + M/2 + D].					
		Average % of insects taken					
Sample no.	% of flowers	N	S	M	D	M + D	Marks
42	1.0	—	—	30	70	100	85
	0.75	—	—	40	60	100	80
	0.50	10	20	25	45	70	62½
	0.35	25	25	30	20	50	41
	0.20	65	20	10	5	15	15
	0.10	90	10	—	—	0	0
	0.075	100	—	—	—	0	0
	82	0.50	—	—	—	100	100
0.35		—	—	5	95	100	97½
0.20		—	—	15	85	100	92½
0.10		42*	26	26	6	32	24
0.075		50	40	10	—	10	15
0.05		100	—	—	—	0	0
L.M.		0.20	—	—	—	100	100
	0.10	—	—	15	85	100	92½
	0.075	—	15	30	55	85	74
	0.05	20	50	10	20	30	37½
	0.025	45	25	10	20	30	31
	0.01	100	—	—	—	0	0
	Sw.	0.35	—	—	—	100	100
0.20		—	—	5	95	100	97½
0.10		—	—	10	90	100	95
0.075		—	—	25	75	100	87½
0.05		—	15	25	60	85	76½
0.025		45	20	25	10	35	27½
0.10		90	5	5	—	5	4
0.005		100	—	—	—	0	0
Controls:							
Saponin	0.5 %	95	—	—	5	5	5
Saponin-alcohol		90	3	3	3	6	4½

\* Nine insects sprayed in one test.

The number of insects used was not sufficiently large to state with precision the concentration giving approximately 50 per cent. of moribund and dead (the best for purposes of comparison), and although we are unable to state whether the toxicity differences found between samples labelled Sw. and L.M. are completely significant, the insects were of sufficiently good quality and the data sound enough to indicate that the toxicities run in the same order as the pyrethrin contents, namely:

Sw. > L.M. > No. 82 > No. 42.

We should tentatively deduce that the 50 per cent. mortality points would be at the following concentrations in terms of the percentage weight in terms of the flowers: Sw. between 0.025 and 0.05 per cent.; L.M. between 0.05 and 0.075; No. 82 between 0.1 and 0.2; No. 42 between 0.35 and 0.5, but in each case rather nearer the lower value. The total pyrethrin contents in percentages run: Sw. 1.98-2.15; L.M. 1.5-1.65; No. 82, 0.92-0.99; No. 42, 0.39-0.44; the order of the percentage contents of pyrethrin I was: Sw. 1.13; L.M. 0.66-0.74; No. 82, 0.39; No. 42, 0.20. There is, therefore, a fairly good concordance between toxicity trials and the analytical tests, but the data cannot be used for determining whether the pyrethrin I content alone is adequate for determining the value of a sample.

Close examination of the data shows that sample No. 42 is not quite as toxic as the analytical data would lead us to expect. There are some grounds for suspecting that, in this case, there has been some loss of toxicity, as the sample (Dalmatian) was derived from the harvest of the year 1926 and was much older than the others tested. If this loss of toxicity be actual, it points to the conclusion that none of the analytical methods available are capable of completely ascertaining its magnitude, and that for this purpose further investigation is required.

#### RAPID METHOD OF EVALUATION OF PYRETHRUM EMPLOYING SMALL QUANTITIES OF MATERIAL.

We have shown above in some detail that, for samples containing high contents of the pyrethrins, some little difficulty may be experienced in the analysis, and we have suggested certain slight modifications to meet these causes, but it was felt that a rapid method of evaluation, employing small quantities of the material, would be of value in providing an index to the amount of sample to be taken for subsequent critical evaluation by the acid method. Furthermore, the possession of

a method of estimating the poisons in a single flower head was regarded as desirable before the physiological and genetical relationships of the pyrethrum plant could be satisfactorily investigated.

The valuable method suggested by Gnadinger and Cori is based upon the reducing properties of the pyrethrins to copper alkaline tartrate solutions. It requires, however, too great a weight of material to be applicable to the analysis of minute amounts of material. During the course of this work we attempted to adapt the method devised by Schaffer and Hartmann for the estimation of small amounts of copper-reducing sugars, and although a certain degree of success was achieved, the titration end-point proved rather unsatisfactory, and we were led to explore other means of analysis. An adaptation of the Hagedorn and Jensen technique as used for the estimation of blood sugar<sup>(3)</sup> was then investigated, and this method was found to be sufficiently sensitive to detect, quantitatively, small amounts of pyrethrins. Partial reduction of a standard alkaline potassium ferricyanide solution is effected by means of the ketone group of the pyrethrolone fraction of the pyrethrin molecule, the degree of reduction being given by the estimation of the amount of ferricyanide present before and after the reaction. This is effected by liberating the iodine equivalent of the ferricyanide, and titrating with standard thiosulphate solution. Graphs have been constructed giving the relationship between the amount of ferricyanide reduced, as expressed in c.c. of *N*/200 thiosulphate, and the mg. of pyrethrins I and II in an aliquot portion of the final pyrethrin extract. Experimental methods have been worked out to enable the estimation of the poisons in:

(a) 0.5 gm. of powdered material.

(b) A single flower head (approx. 0.1 gm. material).

These will be referred to as Methods A and B respectively. It is perhaps necessary to point out that considerable care and some practice in the technique are required for the satisfactory application of both these methods.

#### *Method A.*

The following solutions are required, employing in all cases A.R. quality chemicals:

##### 1. Alkaline ferricyanide solution:

Potassium ferricyanide	1.649 gm.
Anhydrous sodium carbonate	28.6 „
made up to 1 litre with distilled water.	

## 2. Ferrocyanide precipitant:

Potassium iodide	5 gm.
Hydrated zinc sulphate	10 „
Sodium chloride	50 „
in 200 c.c. of solution.	

3. Acetic acid. 3 c.c. glacial acid in 100 c.c. of solution.

4. Hydrated zinc sulphate 1.25 gm. in 250 c.c. of solution.

5. *N*/10 sodium hydroxide.

6. Starch solution. 1 gm. soluble starch in 100 c.c. of solution and saturated with 20 gm. sodium chloride.

7. Aldehyde-free absolute alcohol.

Absolute alcohol, containing 5 gm. *m*-phenylene diamine hydrochloride per litre, is allowed to stand 24 hours with frequent shaking, refluxed for 8 hours and then distilled, being kept in small well-filled stoppered bottles in a dark place, as suggested by Gnädinger and Corl(1).

8. *N*/200 thiosulphate. 1.243 gm. per litre, using boiled-out distilled water, and protected with a soda-lime tube. The thiosulphate solution is most readily standardised by titration of a *N*/200 solution of potassium iodate, prepared by weighing exactly 0.1784 gm. of the dried solid (A.R. quality) and making up to a volume of 1 litre in a standardised volumetric flask. 10.0 c.c. are accurately delivered into a small conical flask by means of a carefully calibrated pipette, employing a definite drainage period. Addition of 5 c.c. of 2 per cent. potassium iodide solution, followed by 3.0 c.c. of 3 per cent. acetic acid solution, liberates the iodine equivalent of the potassium iodate, which is then titrated with the thio-sulphate solution.

*Preparation of pyrethrin extract.* 0.5 gm. of material is extracted with petroleum ether (B.P. below 40° C.) in a Soxhlet apparatus, and the solvent removed by gentle warming, in a current of CO<sub>2</sub>, final traces being removed in a vacuum desiccator. The residue is extracted on a boiling water bath with five successive portions of 4 c.c. each of aldehyde-free absolute alcohol. To the hot solution 1 c.c. of *N*/10 NaOH, and then 4 c.c. of dilute zinc sulphate are added, the solutions mixed, and warmed on the water bath for a few minutes, precipitation of proteins being thus effected. The solution is cooled to 20° C., made up to 25 c.c. with aldehyde-free alcohol, shaken and allowed to stand. The final protein-free extract is obtained by filtering through a small Whatman No. 1 filter paper. For the estimation, 2 c.c. of extract (delivered from a fine nozzle and standardised pipette) are heated for a definite time in a boiling

water bath with 10.0 c.c. of alkaline ferricyanide solution A (accurately delivered), in a Folin tube<sup>1</sup>. The solution is then cooled, washed into a small conical flask, and excess of ferrocyanide precipitant immediately added. The iodine equivalent of the remaining ferricyanide is liberated

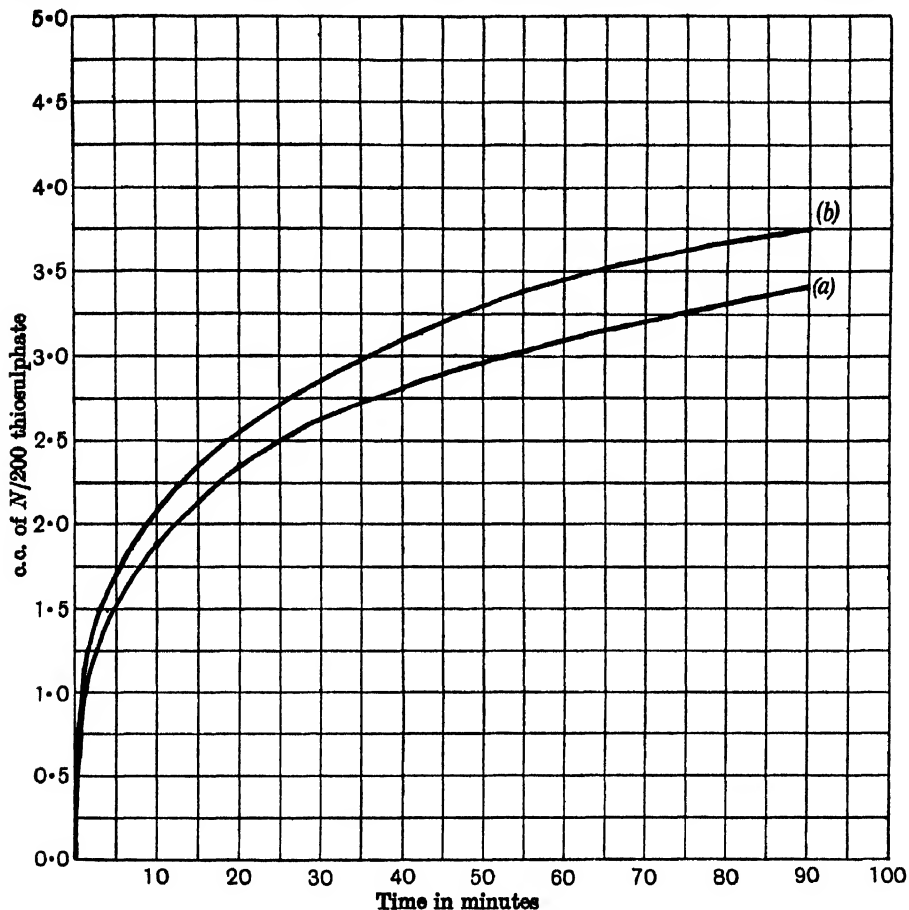


Fig. 1. Graphs a, b

by addition of 10 c.c. of 3 per cent. acetic acid, and, by means of a micro-burette, titrated with thiosulphate, using starch as indicator towards the end of the reaction.

A blank determination on 10 c.c. of ferricyanide is carried out, using 2 c.c. of 80 per cent. alcohol. We have preferred to prepare the alcohol in a way exactly similar to the preparation of the pyrethrin extract, by

<sup>1</sup> After experimenting with test-tubes of various dimensions we found the Folin bulb and tube modified by Gnadinger and Corl(1) a suitable vessel in which to carry out the reduction. The dimensions are: bulb to contain 15.5 c.c. with narrow portion of tube 4 cm. long, and with an internal diameter of 6-7 mm.

addition of sodium hydroxide and zinc sulphate to 20 c.c. followed by heating, and filtering after making up to 25 c.c. We have, however, found no significant differences between blank determination on 10 c.c. of reagent when using this treated alcohol, and that prepared directly from aldehyde-free absolute alcohol.

From the difference in the thiosulphate titrations the amount of pyrethrins in 2 c.c. of the filtrate may be read directly from graph A (Fig. 2). The pyrethrins in 25 c.c. of the extract and, therefore, in 0.5 gm. of material used, can thus be readily obtained.

*Period of heating necessary for the oxidation of the pyrethrins.* An extract of pyrethrum, containing a known amount of poisons, was obtained, and portions of 2 c.c. each heated in a boiling water bath for intervals of 10, 20, 30 minutes, etc. up to  $1\frac{1}{2}$  hours. The amount of reduction was estimated in each case and correlated with time of heating. The results are expressed in Fig. 1 (a). It will be seen that most of the pyrethrin oxidation is effected in the first 45 minutes, there being after this a further slight reduction, approximately constant in amount over further equal intervals of time. This effect was still observed after heating for a period of 2–3 hours, and was not reduced by precipitation of resin acids as their barium salts, together with proteins in the preparation of the extract, by employing barium hydroxide and zinc sulphate. It is probably due to slight interaction between the ferricyanide and alcohol upon prolonged heating. The convenient period of 45 minutes was, therefore, taken as being the minimum time for oxidation of the pyrethrins under these conditions.

*Standardisation of graph.* A sample of pyrethrum was analysed by the acid and Gnadinger and Corl methods; the results obtained were as follows:

Acid method:

(1) Pyrethrin I, 0.77 per cent.; pyrethrin II, 0.76 per cent.

Total pyrethrins, 1.53 per cent.

(2) Pyrethrin I, 0.74 per cent.; pyrethrin II, 0.73 per cent.

Total pyrethrins, 1.47 per cent.

Gnadinger and Corl method:

(1) Total pyrethrins, 1.49 per cent.

The sample had the advantage of being very rich and of containing the two active principles in equal proportions; a mean value of 1.5 per cent. total pyrethrins was taken. It was extracted with petroleum ether (B.P. below 40° C.) and freed from proteins in the way outlined above. A number of dilutions of known strength were prepared, and 2 c.c.



portions of each heated with 10 c.c. of alkaline ferricyanide for 45 minutes in a boiling water bath. The amount of reduction was estimated in each case, and, expressed in terms of  $N/200$  thiosulphate, was plotted against the mg. of pyrethrins contained in 2 c.c. of each extract (Graph A, Fig. 2). The amounts of pyrethrins per 2 c.c. of extract ranged from 0.16 mg.

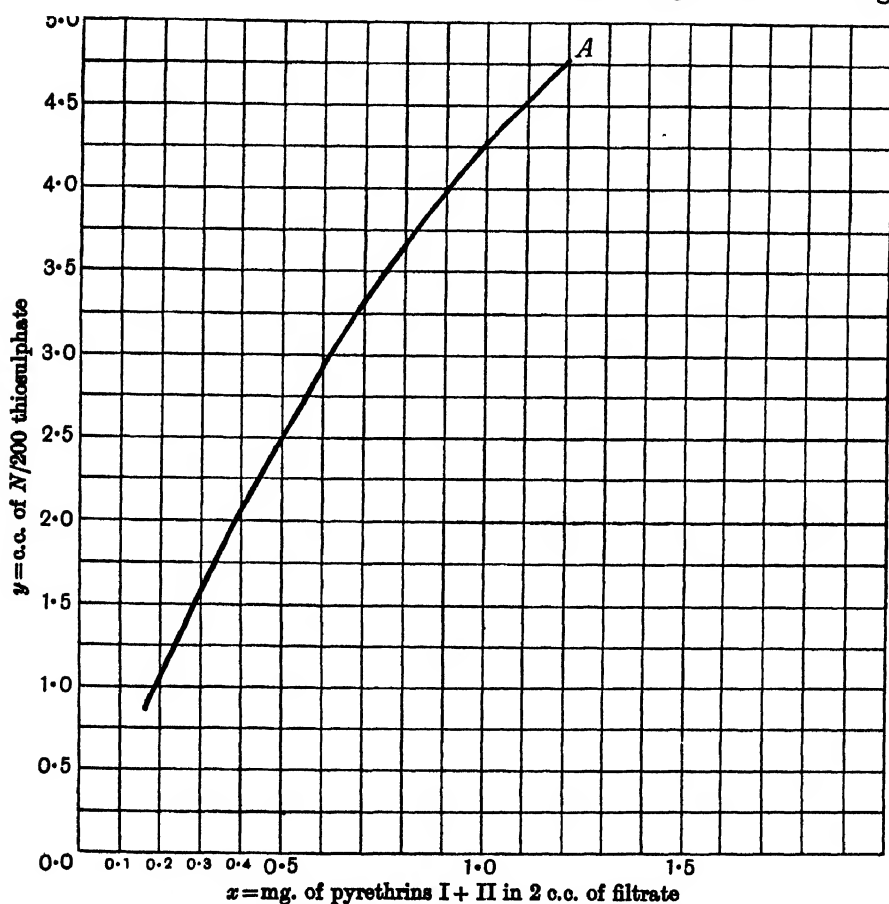


Fig. 2. Graph A. 0.5 gm. of material; equation  $y = -0.067 + 5.969x - 1.616x^2$

to 1.2 mg., giving a range of values for the pyrethrin content, when expressed on 0.5 gm. of material, of from 0.40 to 3 per cent. The points as determined experimentally were found to be close to the parabolic curve as expressed by the equation

$$y = -0.067 + 5.969x - 1.616x^2,$$

where

$y$  = c.c. of  $N/200$  thiosulphate equivalent to the reduction,  
 $x$  = mg. of pyrethrins I and II in 2 c.c. of filtrate.

The values for  $x$  and  $y$  used in computing the graph are given below:

When	$x = 0.16$	$y = 0.864$
	0.30	1.578
	0.40	2.062
	0.48	2.426
	0.60	2.932
	0.69	3.282
	0.86	3.871
	1.00	4.286
	1.20	4.769

Results obtained by using this method are summarised in Table IV, together with the analyses carried out by the acid method.

Table IV. *Analytical results by Method A.*

Sample no.	Acid method			Total pyrethrins (%)	
	Amount taken (gm.)	Pyrethrin I (%)	Pyrethrin II (%)	Acid method	Ferricyanide method A
42	10.0	0.20	0.24	0.44	0.40
E. 12 buttons	5.0	0.23	0.30	0.53	0.55
E. 12 $\frac{3}{4}$ open	5.0	0.32	0.37	0.69	0.69
E. 8 (1)	5.0	0.30	0.38	0.68	0.78*
(2)	2.5	0.53	0.46	0.99	0.90
E. 6	5.0	0.29	0.45	0.74	0.80
D.L. (1)	5.0	0.24	0.32	0.56	0.70
(2)	5.0	0.23	0.36	0.59	—
E. 5 (1)	5.0	0.45	0.46	0.91	0.90
(2)	5.0	0.44	0.51	0.95	—
F. 12	5.0	0.42	0.57	0.99	1.00
F. 10	2.5	0.39	0.58	0.97	1.12
E. 10 (1)	5.0	0.44	0.56	1.00	1.08
(2)	2.5	0.47	0.58	1.05	—
F. 2	2.5	0.60	0.56	1.16	1.10
E. 3	2.5	0.50	0.62	1.12	1.25
E. 7	5.0	0.56	0.61	1.17	1.25
E. 4	2.5	0.39	0.79	1.18	1.28
E. 9	5.0	0.62	0.56	1.18	1.30
F. 4	2.5	0.70	0.69	1.39	1.35
F. 3	5.0	0.56	0.65	1.21	1.40
E. 11 (1)	5.0	0.90	0.70	1.60	1.65
(2)	2.5	0.95	0.77	1.72	—
D. 12	2.5	0.41	0.80	1.21	1.40
D. 11	2.5	0.23	1.18	1.41	1.63
F. 8	2.5	0.78	0.95	1.73	1.73
G. 8	2.5	0.95	0.95	1.90	2.10
F. 11	2.5	1.32	0.86	2.18	1.93
L.M. (1)	5.0	0.67	0.87	1.54	1.58
(2)	5.0	0.74	0.84	1.58	1.58
(3)	2.5	0.66	0.99	1.65	—
Sw.	5.0	1.13	1.02	2.15	2.05

\* The discrepancies noted in E. 8 were largely due to the shaking-out of the sample (p. 121).

It will be seen that fairly good agreements hold for analyses over a range of pyrethrin contents extending from 0.40 to 2.0 per cent., particularly close agreements being observed in the sample L.M., when two observers independently obtained 1.58 per cent., a value agreeing with the average figure by the acid method. The greatest observed discrepancies occur in the F. 11, D. 11 and D.L. samples. In the first of these, however, the pyrethrin content was remarkably high, while the D.L. sample was of unknown commercial origin, and did not possess the odour characteristic of pyrethrum flowers. In this case, however, the result by the ferricyanide method is seen to be in excess of the acid method figure, but agrees with the value given by the Gnadinger and Corl method. We should state that the method has not been primarily devised for the detection of adulteration, but for the evaluation of unadulterated samples of known origin. Where approximate rapid analyses with small quantities of material were required, the method has been of value. In its present form, however, it does not appear applicable to the evaluation of flower heads in the very early stages of development (minute buds). In some of these cases we have recorded results considerably in excess of those given by the acid method. The hypothesis that in some cases reducing bodies, similar to pyrethrolone but not linked with the pyrethrin acids, may exist in the plant has already been tentatively suggested, and indications that this may be the case are particularly strong when dealing with flowers in the early stages of development.

### *Method B.*

*Evaluation of single flower heads.* Individual flower heads selected at random from an air-dried English-grown sample were weighed, the results being as follows: 0.16, 0.12, 0.14, 0.09, 0.10, 0.13, 0.12 gm.

It, therefore, seemed useful to attempt to adapt the method to estimate the active principles in weights round about 0.1 gm. The only modification in the solutions used is in the ferricyanide reagent.

#### 1. Alkaline ferricyanide solution B:

3.30 gm. potassium ferricyanide.

57.20 gm. anhydrous sodium carbonate in 1 litre of solution.

The other reagents used are as in Method A, where 0.5 gm. of material is taken. In the actual analysis, after precipitation of the protein as in Method A, 10 c.c. of the protein-free extract of the pyrethrins are taken, together with 5 c.c. of the alkaline ferricyanide solution B. In this case, heating is carried out in a bath, fitted with a stirrer, and controlled at 78° C. As with Method A, a period of heating of 45 minutes

is employed, since it was observed that, under these conditions, most of the oxidation is effected in the first 45 minutes (see Graph *b*, Fig. 1). A lagging effect, similar to that observed in standardising the time of heating in Method A, was also observed.

Blank determinations on 5 c.c. of the ferricyanide reagent are carried out as before, using 10 c.c. of aldehyde-free alcohol, treated as described in Method A. In Graph B (Fig. 3), standardised in a similar way to Graph A, is recorded correlation data between the amount of reduction, expressed in c.c. of *N*/200 thiosulphate, and the mg. of pyrethrins I and II in 10 c.c. of protein-free extract. From the differences in the readings observed between the blank and the test titrations a simple calculation gives the total pyrethrin content of the sample.

The experimental values were found to fit closely the curve expressed by the equation

$$y = -0.125 + 4.143x - 0.318x^2,$$

where  $y$  = c.c. of *N*/200 thiosulphate,

$x$  = mg. of pyrethrins I and II in 10 c.c. of extract.

The values used in constructing the curve are as follows:

When	$x = 0.15$	$y = 0.489$
	0.30	1.089
	0.44	1.636
	0.538	2.012
	0.60	2.246
	0.867	3.228
	1.20	4.389

Results obtained by analysis of 0.1 gm. of sample, together with results given by the acid method, are summarised in Table V.

Table V. *Analytical results by Method B.*

Sample no.	Acid method			Total pyrethrins (%)	
	Amount taken (gm.)	Pyrethrin I (%)	Pyrethrin II (%)	Acid method	Ferricyanide method B
42	10.0	0.20	0.24	0.44	0.50
82	5.0	0.38	0.60	0.98	1.00
E. 8	5.0	0.30	0.38	0.68	0.70
E. 6	5.0	0.29	0.45	0.74	0.73
F. 12	5.0	0.42	0.57	0.99	0.98
E. 3	2.5	0.50	0.60	1.10	1.08
E. 7	5.0	0.56	0.61	1.17	1.10
L.M. (1)	5.0	0.67	0.87	1.54	1.60
(2)	5.0	0.74	0.84	1.58	—
(3)	2.5	0.66	0.99	1.65	—
F. 11	2.5	1.32	0.86	2.18	1.85

The variation in pyrethrin content in flower heads from one plant, at different stages of development, was next investigated. Individual flower heads, showing successive stages from the half open to the fully overblown conditions, were taken. The stalks were completely removed, the heads finely ground, and dried in an oven at 100° C. for 2 hours,

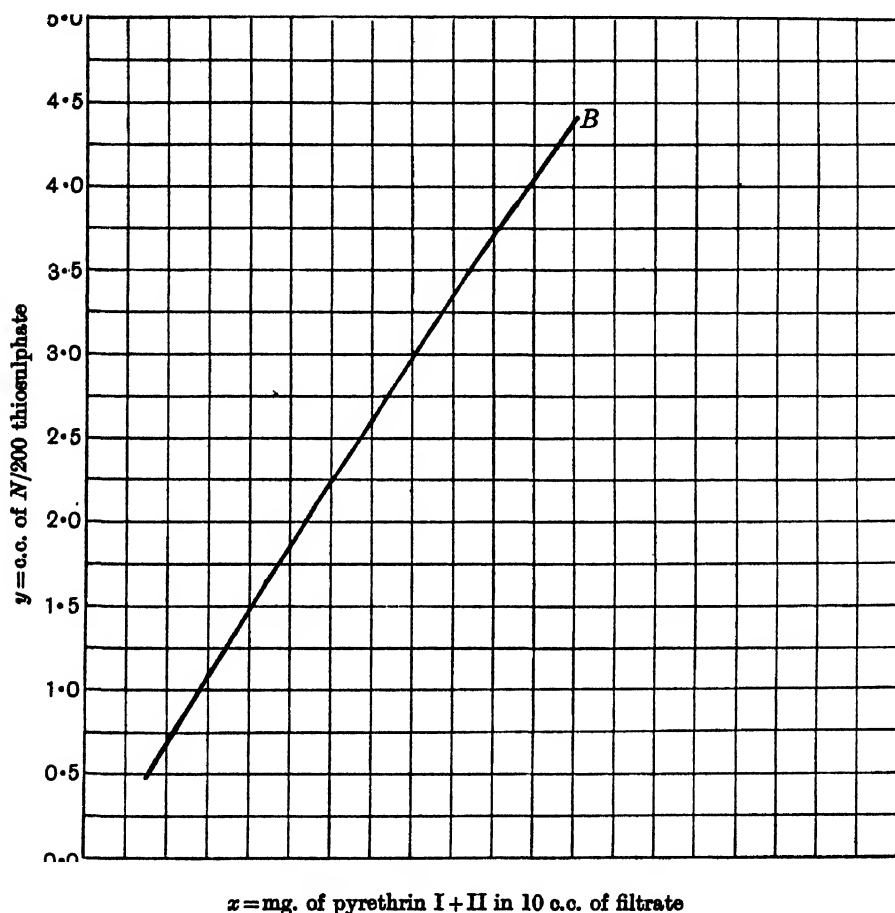


Fig. 3. Graph B. 0.1 gm. of material; equation  $y = -0.125 + 4.143x - 0.318x^2$

weighed accurately, and extracted with petroleum ether after grinding with sand. The residues were taken up with aldehyde-free alcohol, the proteins precipitated, and estimation of the active principles carried out. The results, given in Table VI, were expressed both as mg. of total pyrethrins per flower head and as their percentage content.

These results lend support to the observation to be recorded elsewhere that a significant drop in the pyrethrin content occurs soon after

the final stage of maturity has been reached, *i.e.* when all the disc florets are in an open state. This fall in poison content appears to commence with the first appearance of discoloration of the petals. It will be seen from Table VI that, when expressed both as weight per flower head, and as percentage, the maximum content coincides with the opening of all the disc florets, but, in contradistinction with the fall in percentage pyrethrin content between the fully opened and overblown flower heads, if the pyrethrins are expressed as mg. per head the difference is scarcely significant.

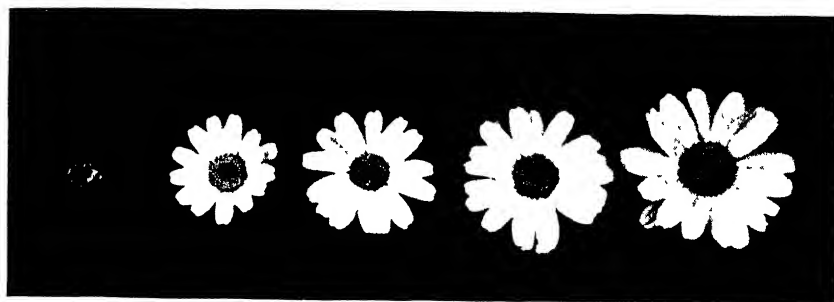
Table VI. *Relationship between stage of development of flowers and pyrethrin content.*

Stage of development	Weight of flower head (gm.)	Total vol. of extract (c.c.)	Pyrethrins per head (mg.)	Pyrethrins per head (%)
Half open	0.0604	25	0.87	1.44
Three-quarters open	0.0848	25	1.18	1.39
First row disc florets open	0.1070	25	1.60	1.50
Half disc florets open	0.1388	25	2.16	1.56
All disc florets open	0.1918	50	3.15	1.64
Overblown	0.2805	50	2.80	1.00

We have found that some considerable confusion exists in the terminology attached to the different stages of development of pyrethrum flower heads. In order to standardise this as far as possible, we have taken as "half open flowers" those showing a tubular arrangement of the petals. Flowers showing one or more of the outer rings of disc florets open we have termed "fully open," while intermediate stages in which the petals are seen expanding, or fully expanded but with no disc florets open, we have designated "three-quarters open." Individual flower heads showing these various stages were taken and photographed.

In Plate I (a) and (b), from 1 to 5, are shown respectively half open flowers, three-quarters open flowers and flowers showing the first row of disc florets open, approximately half the disc florets open, and practically all the disc florets open. Thus under our terminology, flowers 3, 4 and 5 are fully open. It is seen from Table VI that the "fully open" flowers all have the pyrethrin contents of the same order, *i.e.* in the region of 1.5 per cent.

We desire to express our indebtedness to Miss A. M. Webster of the Statistical Department for determining the equations of Graphs A and B.



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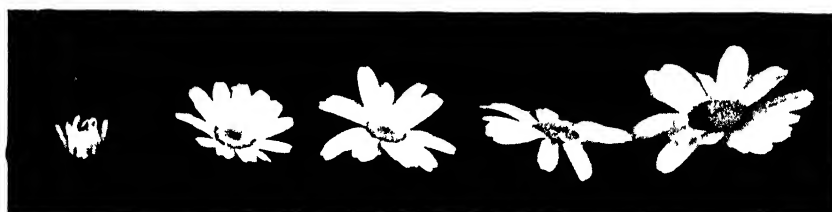
2

3

4

5

(a)



1

2

3

4

5

(b)





## SUMMARY.

1. The analytical methods of Tattersfield, Hobson and Gimingham (6) and Gnadinger and Corl (1) for the determination of the pyrethrins in pyrethrum flowers are compared, and certain modifications in technique suggested.

2. Good concordances have been obtained between analytical data and insecticidal tests employing *Aphis rumicis*.

3. A new method for the rapid and approximate evaluation of unadulterated samples, employing small quantities of material, is described.

4. Observations on the pyrethrin content of individual flowers in the various stages of development are recorded, making use of a modification of the method indicated under (3).

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# EXTRACTS OF PYRETHRUM: PERMANENCE OF TOXICITY AND STABILITY OF EMULSIONS

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(With 5 Text-figures.)

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## INTRODUCTION.

UNTIL quite recent times the flowers of pyrethrum (*Chrysanthemum cinerariaefolium*) were generally used for insecticidal purposes as a finely ground powder. Considering the small amount of the active principles

present in the flowers, it is surprising that this method was as effective as it was generally found to be. That the full activity of the poisons did not come into play can hardly be a matter of doubt, and the attempts in more recent years to prepare and employ extracts for spraying purposes has probably led to a much wider field of usefulness for this valuable insecticide.

Until the structure of the two pyrethrins had been elucidated by Staudinger and Ruzicka<sup>(8)</sup>, who have shown them to be esters and liable to decomposition or chemical change detrimental to the insecticidal action, the introduction of rational principles to the making of such spray fluids was hardly to be expected. Staudinger and Harder<sup>(7)</sup> indicated that loss of toxicity might occur in the alcoholic-soap extracts (known in France and Switzerland as Savon-pyrèthre) by hydrolysis, as neither the acid nor the alcohol portions of the esters are materially toxic to insects, and in addition by alcohol-radical exchanges particularly in the presence of methyl alcohol. Thus a careful choice of solvents and emulsifier is a matter of importance. More inert solvents than alcohol and less reactive emulsifiers and wetters than soap have therefore been suggested. This paper is devoted to an examination of the problem of preparing mixtures, in which the poisons are stable and from which sufficiently stable emulsions can be simply made. It is obvious, however, that only a limited number of the many possible combinations could be examined and therefore a selected number of extracts were prepared.

There are several considerations to be taken into account when framing experiments for the study of this problem: (1) the poisons should not undergo chemical change within a reasonable time, (2) the extract should readily mix with water to form a suitable fluid, *i.e.* the emulsions should be stable enough to be conveniently applied, (3) the spray should not be injurious to foliage.

(1) The active principles of pyrethrum are esters. *Prima facie* it would, therefore, appear that the use of strong alkalis in the mixtures and spray fluids would have to be limited and the extent to which they could be used with safety determined. In addition, the employment of strong emulsions did not seem desirable, and after some experiments on the emulsification of strong pyrethrum-petroleum extracts we decided to devote attention chiefly to petroleum and alcoholic extracts and to the so-called miscible oil preparations, *i.e.* to clear oils which, while they contain a minimum amount of water and are insoluble therein, nevertheless will, under suitable conditions, mix fairly readily with water as oil-in-water emulsions.

(2) Emulsions or extracts of pyrethrum in water-soluble solvents can, in general, be readily diluted. It was, however, found early in this work that some constituent of pyrethrum flowers had a de-emulsifying effect and that extracts made with light petroleum oils and an emulsifying agent could not be worked into the form of stable emulsions with anything like the same ease as the corresponding inert petroleum oil alone. Some of the conditions involved in emulsifying pyrethrum extracts are the subject of a further communication by one of us (R.P.H.). Pyrethrum emulsions might become relatively unstable during transport, and vibration, variation in temperature, etc., might cause a separation of the dispersed phase or a reversion to a water-in-oil condition. Cogency is thus given to the argument in favour of devoting greater attention to the miscible oils, which, being clear solutions, are generally not sensitive to external conditions except that of temperature which can be readily tested in the laboratory.

(3) The use of certain organic solvents in conjunction with pyrethrum is further justified if it can be shown that they too have an insecticidal value or enhance that of pyrethrum. That this is so, particularly in the case of petroleum solvents, has been suspected for some time, and our data lead to a similar conclusion. On the other hand, considerable care has to be exercised in using petroleum products upon foliage. Light fractions, if used above certain concentrations, may have an immediate serious effect, and heavy fractions a delayed but none the less destructive action. The oil used should be of relatively high purity and the concentration in the spray fluid well within the margin of safety. The following is a summary of certain of the findings of deOng, Knight and Chamberlin<sup>(5)</sup>. (a) Non-viscous oils of a low boiling point, such as kerosenes, are safer in use on the tree than those of high-boiling points, but are unsatisfactory as scalecides because of relatively low toxicity combined with high volatility. (b) Highly refined, white lubricating oils are probably the most advisable for use on citrus trees, especially at summer temperatures. Oils of low viscosity are apparently safer to use on trees than those of high viscosity. (c) Severe injury to the citrus tree from the use of lubricating oil is associated with the presence of a high percentage of unsaturated hydrocarbons. (d) Gross symptoms of injury to citrus trees may result from the use of unrefined petroleum oils, including defoliation, fruit spotting, dropping and the killing of twigs and branches. (e) A quick-breaking emulsion utilises to the maximum degree the insecticidal agent. deOng<sup>(6)</sup> has also given specifications for petroleum oils to be used on plants. It appears, therefore, that the use of petroleum

in a spray fluid would tend to lower the concentration of the more costly pyrethrum necessary to kill and the effect would be the greater the larger the amount of it that could be employed commensurate with safety to the tree or plant. This amount, however, would be dependent on the petroleum fraction used and its freedom from deleterious substances.

#### PERMANENCE OF THE ACTIVE PRINCIPLES.

As the active principles of pyrethrum are complex esters and are supposed readily to undergo chemical change and hydrolysis, the results of which lessen toxicity, some attention had to be given to the degree of stability of the poisons in certain solvents and in the presence of certain emulsifiers. It was decided to carry out these experiments under two sets of conditions, at the laboratory temperature and at one corresponding approximately to the mean temperature in tropical countries; 28 to 30° C. was chosen as one that could be readily maintained for long periods. Preparations of pyrethrum were, therefore, made and divided into two portions, one of which was allowed to stand in the laboratory, the other placed in a stoppered tube and kept in an incubator adjusted to 28 to 30° C. Attempts were made as far as possible to adjust the concentration of the active principles so that the amounts of solvent and adjuvant would be insufficient when used by themselves to have any serious toxic action on the insects used. In some of the tests, petroleum ether rather than the higher boiling fractions of petroleum was used as giving a greater margin of safety in this regard. Permanent emulsification of petroleum ether extracts of pyrethrum is more difficult than those made with the higher boiling fractions, and this leads to some difficulty in interpreting the results, when no emulsifier has been incorporated.

As our stock of *Aphis rumicis*, the test insect employed, had to be started afresh during the year 1929 from a wild colony, our insects cannot be regarded as standardised to the same extent as in the previous years; and as the experiments have been spread intermittently over 2 years, the results have to be judged with some care in that cross-comparisons from data obtained on different dates should be made with caution, and the effects produced should be compared as far as possible with those obtained on the same day. Owing to variations in insect resistance on different dates, the control tests did not always show absolute blanks, due in certain cases to some slight toxic action of some of the adjuvants; for comparative tests, however, amongst preparations sufficiently alike in composition, this need not lead to erroneous conclusions.



The spray trials were carried out in the way and by the use of the apparatus previously described(11). Observations on the effects of spraying were carried on for 3 days, the results at the end of 3 days being taken except where meteorological conditions were such as to lead to heavy mortalities in the controls, in which case the results at the end of the second day are given (only necessary in one case). The toxic effects were expressed by giving the percentage number of insects falling into the four categories: (1) those not affected, (2) those slightly affected, (3) those moribund, (4) those apparently dead. The examination of the data expressed fully, although preferable as showing grading effects, would make the tables unduly long and cumbersome and therefore in general we have abbreviated them by giving the collected percentages of moribund and apparently dead insects for each concentration.

*Pyrethrum flowers.* A sample of the flowers, taken from a plot in Harpenden in 1928, was divided and one half ground; both the whole and the ground halves were again divided and each portion placed in a large tube so that it was half-filled by the material, the tubes were then closed by tightly fitting rubber stoppers. One tube of the ground and one of the whole heads were placed in an incubator and kept at 28° C., the other two being allowed to stand at laboratory temperature. In addition a sample of the ground material was spread in a thin layer in a Petri dish and placed in the incubator. Eighteen months later all of the samples were extracted with absolute alcohol, diluted with a 0.5 per cent. solution of saponin and their toxicities to *A. rumicis* determined. The results are presented in Table I. The data given in Table I show that there is little difference after 18 months between the toxicities of the two samples kept in tubes at 28° C. and those allowed to stand at room temperature. In addition the table includes data for a sample of the same pyrethrum tested shortly after harvesting. Too close a comparison with the results obtained for this sample and the above must not be attempted, as it was extracted in a different way and the concentrations tested are slightly different and moreover 2 years have elapsed between the respective tests, but it appears safe to conclude that the results given by it are of the same order and that the stored samples have stood up well to the effect of time and temperature. This, however, only applies to samples stored in closed tubes, for the sample exposed in a thin layer at 28° C. has lost the greater part of its toxicity in 18 months. In addition a commercially prepared pyrethrum dust was found to lose a greater proportion of its insecticidal properties when exposed

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in a thin layer at room temperature for a fortnight. As the air in the incubator and the room were comparatively dry, the question arises whether the loss of toxicity could be due to hydrolysis, and it is reasonable to conclude that other factors may be involved.

Table I. *Permanence of toxicity of pyrethrum.*

Series A stood as flowers. Series B stood as 10 % alcoholic extracts of flowers.

Test-subject *Aphis rumicis*.

		Percentage moribund and apparently dead insects 3 days after spraying at concentrations of			
	Description of test	Percent. flowers ,, pyrethrin I	0.35 0.0018	0.2 0.001	0.1 0.0005
<i>Series A:</i>					
	Unground heads at lab. temp. 2 years stoppered tube		100	90	50
	Unground heads at 28° C. 18-19 months stoppered tube		100	100	80
	Ground heads at lab. temp. 18-19 months stoppered tube		100	100	60
	Ground heads at 28° C. 18-19 months stoppered tube		100	80	70
	Ground heads at 28° C. 18-19 months in a thin layer		40	20	10
	0.5 % saponin solution and alcohol to correspond with		10	0	—
<i>Series B:</i>					
	Stood at lab. temp. as 10 % extract in <i>absolute alcohol</i> 18-19 months		100	100	80
	Stood at 28° C. as 10 % extract in <i>absolute alcohol</i> 18-19 months		100	100	75
	Stood at lab. temp. as 10 % extract in 95 % alcohol 7½ months		100	100	65
	Stood at 28° C. as 10 % extract in 95 % alcohol 7½ months		100	100	60
	Stood at lab. temp. as 10 % extract in 95 % alcohol 18-19 months		100	95	72.5
	Stood at 28° C. as 10 % extract in 95 % alcohol 18-19 months		100	100	65
	0.5 % saponin solution and absolute alcohol to correspond with		10	0	—
	0.5 % saponin solution and 95 % alcohol to correspond with		5	0	—
		Percent. flowers	0.44	0.18	0.13
		,, pyrethrin I	0.0022	0.0009	0.0006
	*Flowers as above immediately after harvesting		100	80	40

\* This test was carried out many months before others in the table, and too close a comparison is not legitimate.

*Alcohol extracts of pyrethrum flowers.* The extraction of pyrethrum by commercial ethyl alcohol has been practised for some years, in many cases the extract being mixed with soap, *e.g.* in savon-pyrèthre. The latter practice has for some time been regarded as unsatisfactory, owing to the risk of hydrolysis or other chemical change of the esters. (Certain

data on the effect of alkalinity on the stability of these compounds are given later.) It was, however, pointed out by Staudinger and Harder (*loc. cit.*) that some risk was involved in the use of alcohol, particularly in the presence of alkali, as the pyrethrins might undergo an alcohol exchange involving loss of toxicity. We have kept for several years the extracts of pyrethrum flowers, prepared by the use of commercial 95 per cent. alcohol which in field practice retained their toxic action down to comparatively low concentrations (0.5 per cent. in terms of flowers). In laboratory trials samples of the same flowers, the toxicity data of which are given in Table I, were extracted by both absolute and commercial 95 per cent. alcohol. The clear solutions, in both cases, were divided and one-half of each kept at laboratory temperature and at 28° C. in tightly corked tubes. They were tested after 8½ months and again after standing a period of 18 months. The data are given in Table I.

It can be concluded from these data, that in temperate climates, alcoholic extracts of pyrethrum can be prepared with some assurance as to the relative permanence of the toxic effect, and that there is surprisingly little loss of toxicity over long periods at temperatures as high as 28° C. Our results, therefore, indicate loss of toxicity to be inconsiderable, provided the extracts be used in a reasonable time after preparation. Mixing with soap solutions should not, however, take place until immediately before spraying is to be carried out. Alcohol extracts have certain advantages in that they are easy to handle and mix with water of all degrees of hardness without difficulty to give an emulsion of great stability.

*Petroleum preparations.* Pyrethrum is often extracted by derivatives of petroleum (*e.g.* petroleum ether) and afterwards made up with kerosene or lighter petroleum fractions. For horticultural work, it may become necessary to prepare oil-in-water emulsions of the petroleum extract at high dilutions with water of different degrees and types of hardness. The petroleum extract can be emulsified to some degree of permanence by vigorous stirring with soap solutions in soft water. As such water is not always available in large quantity and as under certain circumstances the reverted or water-in-oil type of emulsion might inadvertently be made, the addition of certain emulsifiers to the oil is recommended for use—yielding so-called miscible oils.

Miscible oils can be prepared by incorporating with the petroleum extract certain sulphonated oils together with alkali. The degree of the permanence of the active principles in the presence of two sulphonated oils was therefore determined. As the pyrethrin esters seemed more

likely to undergo chemical change, especially hydrolysis, in the presence of alkalies of a high degree of dissociation, ammonia in varying proportions was mixed with one of the sulphonated oils and the tests carried out at laboratory temperatures and at 28 to 30° C.

One of us (R. P. H.) has investigated the effect upon interfacial tension of using buffers of different *pH* with petroleum extracts of pyrethrum<sup>(4)</sup>. He was able to determine the *pH* at which the stream of extract from a dropping pipette failed to form discrete bubbles and noted that some degree of alkalinity in the water, used for dilution of certain of the miscible oil preparations, rendered emulsification much more easy and rapid. A certain number of tests were, therefore, carried out to ascertain how long the pyrethrins would remain active when dispersed in media of different *pH* values (p. 224).

*Petroleum-ether extracts.* Tests were carried out on a 10 per cent. petroleum ether extract of pyrethrum, one portion of which had stood at laboratory temperature and the other at 28° C. for 7½ months and again for a full period of 18 months. The toxicity trials presented very considerable difficulties owing to the separation of the ether from the dilutions with solutions of 0·5 per cent. saponin. There was, however, very little difference in the insecticidal values to be noted between the two samples after 7½ months and again after 18 months—but both samples were less effective at the end of 18 months than they were after 7½ months. It is impossible to state, without further investigation, whether the loss was real or due to a greater insect resistance on the later date, or whether owing to rapid separation the respective concentrations varied on the two dates. It was noted that the sample warmed to 28° C., lost almost the whole of the yellow colour initially characteristic of the extract.

*Water-miscible oils containing Agral W.B.* A series of trials were carried out with an emulsifier known to commerce as Agral W.B. Certain tests were also made with ammonia oleate for purposes of comparison. The former we found could be used with calcium-hard water, whereas ammonium oleate would necessitate the use of soft waters.

A 20 per cent.<sup>1</sup> extract of pyrethrum (in terms of the flower heads) in technical petroleum ether was mixed with a 25 per cent. solution of Agral W.B., in such proportions as to give a 10 per cent.<sup>1</sup> content of Agral W.B. This mixture was allowed to stand for a period of over

<sup>1</sup> The term per cent. throughout this paper usually has the meaning of gm. per 100 c.c.

6 months both at laboratory temperature and at 28–30° C. The samples were then diluted with 0.5 per cent. saponin in water and tested against a freshly prepared mixture. The results are set out in Table II. In addition, this table (series B) contains data for a mixture of a 20 per cent. solution of Agral W.B. to which had been added 0.2 cc. of 0.902 ammonia to remove a little cloudiness.

Table II. *Permanence of toxicity of petroleum-ether extracts of pyrethrum in the presence of Agral W.B.*

Concentrations expressed in terms of flowers. Test subject *Aphis rumicis*.

Preparation and time of standing	Percentage moribund and apparently dead insects 3 days after spraying at concentrations of		
	0.35 %	0.2 %	0.1 %
<i>Series A :</i>			
Mixture containing 12 % pyrethrum flowers and 10 % Agral W.B. Stood at lab. temp. 6 months 21 days	100	100	50
Mixture as above. Stood at 28–30° C. 6 months 21 days	100	100	70
Mixture as above. Freshly prepared ... ..	100	100	30
Control Agral W.B. and solvents to correspond with 20 % pyrethrum extract in petroleum ether (no adjuvants)	10	0	0
	100	80	65
<i>Series B :</i>			
Mixture equivalent to 16 % pyrethrum flowers and 20 % Agral W.B. with 0.2 c.c./100 c.c. of ammonia (0.9). Stood at lab. temp. 7 months 3 days	100	50	10
Mixture as above. Stood at 28–30° C. 7 months 3 days	100	70	10
Mixture as above. Freshly prepared ... ..	90	80	30
Control Agral W.B. and solvents to correspond with	40	30	20

Series A on cooling to 0–3° C. gave a faint precipitate. Series B were clear.

\* Cross references between series A and B should not be made—the insects used for series A were less resistant than those used in series B.

It should be pointed out that comparisons between series A and B cannot be made with advantage, as not only were the mixtures tested on different days but were made up in different ways on different dates. We can deduce from series A of this table that the presence of 10 per cent. of Agral W.B. has little or no harmful effect upon the stability of the active principles of pyrethrum over a period of several months either at laboratory temperature or at 28 to 30° C.

In series B the Agral W.B. at the higher concentration seems to have had a killing effect, due probably to the clogging of the respiratory system; in any case such a result with too high a concentration of a viscous oil is not unlikely to occur. This does not invalidate the deduction from this series that the addition of 20 per cent. Agral W.B. did

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not materially lower the toxicity of the samples that had stood over 7 months either at laboratory temperature or at 28 to 30° C.

For the preliminary tests with Agral W.B. and oleic acid treated with ammonia, Agral W.B. and oleic acid were dissolved in technical petroleum-ether and dry ammonia gas passed until supersaturated. In the case of oleic acid the solution became gelatinous and turbid, and in order to clear, an amount of 95 per cent. alcohol was added. Stock solutions equivalent to approximately 25 per cent. of the original Agral W.B. and oleic acid were made up and 20 c.c. of this added to 30 c.c. of an extract of pyrethrum flowers in technical petroleum-ether, making a mixture equivalent to 12 per cent. pyrethrum in terms of flowers, 10 per cent. of Agral W.B. and of oleic acid. Each mixture was divided into two parts, one being allowed to stand at laboratory temperature and the other at 28 to 30° C. The results obtained with dilutions of these mixtures are given in Table III. Owing to very warm weather prevailing at the time of the tests they could not be carried beyond 2 days without risk of exaggerating the toxic effects, and even at the end of 2 days the lowest concentration in the control test with Agral W.B. shows an apparent toxicity. A sufficient number of insects of good quality were not available to test out extracts made up just prior to the tests. Reference to the table shows that the mixture containing Agral W.B. kept at 28 to 30° C. is not completely toxic at the highest concentration used, in contrast with the sample kept at laboratory temperature. It may be deduced therefore that under tropical temperatures this mixture could not be expected to retain its insecticidal properties for long periods. The treatment is, however, very drastic and the toxicities for both samples proved higher than expected.

Ammonium oleate is itself toxic to *Aphis rumicis*, and the results in the table are therefore difficult to interpret. We consider that the mixtures prepared from dry ammonium oleate are not likely to remain unaltered in toxic properties either in temperate or tropical climates. The sample made from oleic acid exactly neutralised by aqueous ammonia and kept at 28 to 30° C. has suffered hardly a significant loss of toxicity as compared with the samples standing at laboratory temperature. It is difficult to say to what extent the toxicity has been enhanced by the presence of ammonium oleate, or how rapid a loss of toxicity of the pyrethrins would result from its use, but seeing that rather careful adjustment of alcohol to petroleum solvent has to be made to prevent turbidity, particularly in the cold, this emulsifier would have to be used with caution.

Table III. *Permanence of toxicity of pyrethrum extract in presence of Agral W.B. (ammoniated) and ammonium oleate.*Concentration expressed in terms of flowers. Test subject *Aphis rumicis*.

Preparation and time of standing	Percentage moribund and apparently dead insects 2 days after spraying at concentrations of			Effect of cooling to 0-3° C. for 24 hours
	0.35 %	0.2 %	0.1 %	
Extract equivalent to 12 % pyrethrum flowers and 10 % Agral W.B. saturated with dry ammonia gas. Stood at lab. temp. 6 months 3 days	100	40	0	Clear
Extract as above. Stood at 28-30° C. 6 months 3 days	70	40	20	
Agral W.B. and solvents to correspond with	0	0	20	
Extract equivalent to 12 % pyrethrum flowers and 10 % oleic acid saturated with dry ammonia gas. Stood at lab. temp. 6 months 3 days	90	40	40	Separated into two layers
Extract equivalent to 12 % pyrethrum flowers and 10 % oleic acid saturated with dry ammonia gas. Stood at 28-30° C. 6 months 3 days	90	30	10	
Oleic acid saturated with dry NH <sub>3</sub> and solvents to correspond with	20	10	10	
Extract equivalent to 12 % pyrethrum flowers and 10 % oleic acid neutralised with ammonia s.g. 0.9. Stood at lab. temp. 6 months 3 days	100	80	30	Trace of precipitate
Extract equivalent to 12 % pyrethrum flowers and 10 % oleic acid neutralised with ammonia s.g. 0.9. Stood at 28-30° C. 6 months 3 days	90	70	30	
Control. Saponin control 0.5 %. No effect	—	—	—	
Control. Petroleum-ether (comm.) 4 c.c./100 c.c. + 0.5 % saponin solution—20 % dead				
"                    "          2 c.c./100 c.c.		"	"	—no effect
"                    "          1 c.c./100 c.c.		"	"	—10 % dead

A set of experiments was started to ascertain if there was a correlation between any loss of toxicity and the proportion of ammonia to Agral W.B. used. To weighed amounts of Agral W.B. in petroleum-ether were added increasing volumes of strong ammonia solution (s.g. 0.902) to give ratios of  $\frac{0.902 \text{ ammonia (c.c.)}}{\text{Agral W.B. (gm.)}}$  of 0.01, 0.02, 0.05, 0.1, and 0.2; to these solutions were added a 20 per cent. pyrethrum extract in petroleum-ether so as to give 10 gm. of Agral W.B. per 100 c.c. The mixture containing the lowest concentration of ammonia was slightly cloudy immediately after mixing but cleared on standing. The remainder were clear, but the sample containing the highest proportion of ammonia was somewhat viscous. Each mixture was divided, one portion being

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kept at laboratory temperature, the other at 28 to 30° C. in an incubator. Their toxicities were determined 5½ months later, and in one case (0.1 ratio) after 18 months. It would be impossible to insert the full tables of data, and therefore summaries are given in Tables IV and V.

Table IV. *Permanence of toxicity of petroleum extracts of pyrethrum containing 10 per cent. Agral W.B. and varying proportions of ammonia (s.g. 0.9).*

Concentrations expressed in terms of flowers. Test subject *Aphis rumicis*.

Ratio	0.902 ammonia c.c. Agral W.B. gm.	Percentage moribund and apparently dead insects 3 days after spraying at concentrations of			Effect of cooling to 0-3° C. for 24 hours
		0.35 %	0.2 %	0.1 %	
0.01 stood at lab. temp. 5 months 19 days		90	70	10	Clear
0.01 „ 28-30° C. „ „		100	100	40*	
0.02 stood at lab. temp. „ „		100	80	10	Clear
0.02 „ 28-30° C. „ „		100	90	20*	
0.05 stood at lab. temp. „ „		90	70	40	Practically clear
0.05 „ 28-30° C. „ „		90	70	—	(Trace ppt.)
0.1 stood at lab. temp. „ „		100	70	40	Practically clear
0.1 „ 28-30° C. „ „		100	100†	—	(Trace ppt.)
0.2 stood at lab. temp. „ „		100	100	—	Clear
0.2 „ 28-30° C. „ „		100	100	20	
20 % pyrethrum extract in petroleum ether, no Agral W.B. Stood at lab. temp. 5 months 27 days		60	20	—	
0.5 % saponin solution 10 % M. and D.; 0.5 % saponin solution. No M. and D.		—	—	—	
Petroleum ether 2 c.c. in 100 c.c. 0.5 % sa- ponin solution and petroleum ether 1 c.c. in 100 c.c. 0.5 % saponin solution gave no moribund and dead		—	—	—	
<i>Duplicate test after standing 18 months:</i>					
0.1 stood at lab. temp.		95	100	50	
0.1 stood at 28-30° C.		100	95	30	

\* A certain amount of volatilisation of the petroleum-ether had taken place at 28-30° C. and although this was made up by the addition of more petroleum-ether, there would be a slightly higher proportion of the less volatile petroleum derivatives in these samples. It is reasonable to suppose that this would add slightly to the toxicity.

† The whole of these were moribund and not dead. If marks had been awarded this value would have been approximately the same as the corresponding sample kept at laboratory temperature.

In Table IV the sum of the percentages of moribund and dead insects for the concentrations tested is given for each mixture. This does not bring out the gradations in the toxic effects, but it does make possible a simple and fairly accurate comparison. Inspection of the table leads to the deduction that samples heated to 28 to 30° C. have stood as well



as the samples left at laboratory temperature. Indeed, in one or two cases the former appear more toxic. The differences are probably not outside experimental error, but since at 28° C. the light petroleum evaporated to some extent and penetrated quite close-fitting stoppers (the level had to be made up again to the mark) there would be left in the warmed samples a rather larger proportion of the high-boiling fractions of petroleum, which are suspected of being the more toxic than the lower-boiling fractions. The sample, in which the ratio ammonia/Agral W.B. was 0.1, was again tested after standing for a further 12 months (*i.e.* 18 in all). There seems to have been little or no change in the toxicity of the mixture.

Table V. *Permanence of toxicity of pyrethrum extracts with 10 per cent. Agral W.B. (ammoniated).*

(Summary)

N.=not affected; S.=slightly affected; M.=moribund; D.=apparently dead.

Results 3 days after spraying.

Preparation and time of standing	Concentration in terms of flowers %	N. %	S. %	M. %	D. %	M. and D. %	Effect of cooling to 0-3° C. 24 hours
<i>Series A*:</i>							
Extracts in Table III containing 10 % Agral W.B. and varying amounts of ammonia. Stood at lab. temp. 5 months 19 days (mean values)	0.35	—	4	48	48	96	All practically clear
	0.2	16	6	42	36	78	
	0.1	68	10	22	—	22	
Extracts as above but stood at 28-30° C. (mean values)	0.35	2	—	19	79	98	
	0.2	2	6	64	28	92	
	0.1	76	8	14	2	16	
0.5 % saponin solution 2 tests (mean results)	—	95	—	—	—	—	
Petroleum-ether + 0.5 % saponin solution, 3 tests	—	100	—	—	—	—	
<i>Series B:</i>							
Extract equivalent to 12 % pyrethrum flowers and 10 % Agral W.B. (ammoniated). Ratio ammonia (0.9) = 0.2. Stood at Agral W.B. lab. temp. 6 months 13 days	0.35	—	—	10	90	100	Clear
	0.2	—	—	40	60	100	
	0.1	10	10	60	20	80	
Extract as above freshly prepared. Stood 24 hours	0.35	—	—	20	80	100	
	0.2	—	30	30	40	70	
	0.1	30	10	30	30	60	
0.5 % saponin solution, 2 tests (mean)	—	100	—	—	—	—	

\* Cross-comparison between series A and B cannot be made. It was shown by tests with a pyrethrum extract free of adjuvants that the insects used in series B were slightly less resistant than those used for series A.

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In Table V the results given in Table IV for the samples kept at laboratory temperature and those kept at 28 to 30° C. are collected together and averaged. In addition, at a later date, the sample containing the highest ratio of ammonia solution to Agral W.B. kept at laboratory temperature was tested against one freshly prepared. These two series are labelled A and B; they should not be inter-compared, as tests carried out on each day with a 20 per cent. extract of pyrethrum, free of adjuvants, showed that the insects were less resistant in series B than in A. From series A it would appear that the sample kept at 28° C. is not less toxic than the one stored at laboratory temperature. Series B presents a curious problem—the freshly prepared sample is apparently somewhat less toxic than the stored sample. It is highly questionable whether this difference is outside experimental error; it should be pointed out, however, that when the sample of ammoniated Agral W.B. used for the extract which stood for 6 months was prepared, some time elapsed before it was mixed with the pyrethrum extract and the resulting product was viscous; with the sample prepared 24 hours previous to testing, this waiting period was very short and the resulting mixture not so viscous. Complete combination between base and oil may not have taken place and some free ammonia may have had a slightly adverse effect on the toxicity in 24 hours. It would appear advisable to allow the ammonia to stand sufficiently long with the Agral W.B. to ensure complete interaction before mixing with the extract of pyrethrum.

A fair deduction to draw from these data is that Agral W.B. can be mixed with petroleum extracts of pyrethrum with comparative safety, that these mixtures can be stored in temperate climates for some time and for several months under tropical and semi-tropical conditions, without much loss of toxicity. The addition of strong ammonia solution to the Agral W.B. to aid clearing and emulsification, provided that time has been allowed for complete reaction between the two, appears from our data to be comparatively safe, if the proportion of ammonia solution (s.g. about 0.9) to Agral W.B. is not greater than about 1 to 5. These tests only apply to mixtures containing 10 per cent. Agral W.B.

Owing to the low toxicity of petroleum-ether to *A. rumicis*, this solvent was used as the organic solvent in the foregoing experiments. In actual practice this solvent would prove unsuitable, as it is too volatile and inflammable and in addition stable emulsions are not easy to prepare from it. Higher fractions such as refined and semi-refined white spirits, kerosene and refined lubricating oils would prove more

generally useful. It would be necessary, however, to use them at concentrations in the tank at which they would be non-injurious to foliage. We regard a concentration of about 1 per cent. to be in general as high as it is safe to use these oils. Agral W.B. is more readily soluble in the lighter fractions such as refined and semi-refined white spirit (s.g. 0.782 and flash point 94° C.), but the higher fractions would be expected to enhance toxicity to a greater extent.

Table VI. *Permanence of toxicity of pyrethrum extracts in the presence of increasing amounts of ammoniated Agral W.B.*

$$\left( \text{Ratio } \frac{0.9 \text{ ammonia}}{\text{Agral W.B.}} = 0.1 \right).$$

Strong extracts have stood 9 months 1 week except where stated.

Concentrations of extracts sprayed are in terms of pyrethrin I.

Test subject *Aphis rumicis*.

Percentage amount of ammoniated Agral W.B. in pyrethrum extract before dilution	Percentage moribund and apparently dead insects 3 days after spraying at concentrations of		
	0.001 %	0.0005 %	0.00025 %
5 % at lab. temp.	100	90	40
5 % at 28–30° C.	100	80	40
*10 % at lab. temp.	100	100	55
*10 % at 28–30° C.	100	80	60
*10 % prepared 1 month	100	80	40
20 % at lab. temp.	100	100	35
20 % at 28–30° C.	100	90	50
20 % freshly prepared	100	70	20
40 % at lab. temp.	100	100	45
40 % at 28–30° C.	90	70	40
Controls with mixtures as above but without pyrethrum gave blanks			
Duplicates after 8 months' standing:	0.002 %	0.001 %	0.0005 %
10 % at lab. temp.	100	100	60
10 % at 28–30° C.	100	100	70
10 % freshly prepared	100	100	80
Controls	10	—	10

\* These samples were tested a month previously—no significant differences were detected in their toxicities, the data are given lower in table.

*Note.* The above mixtures when cooled to 0° C. gave a slight deposit after 2 days. In the case of the mixture containing 40 % ammoniated Agral W.B. there was rather much deposit.

A series of tests was made in which the ammoniated Agral W.B. was used in the successively increasing amounts of 5, 10, 20 and 40 per cent. The ratio of 0.9 ammonia (c.c.) to Agral W.B. (gm.) was in each case 0.1, semi-refined white spirit was used as solvent and the concentration of the pyrethrum extract adjusted to bring the content of

pyrethrin I to 0.5 per cent. Each preparation was divided into two parts, one of which was kept at room temperature and the other allowed to stand at 28° C. for just over 9 months. They were then tested for their toxicity to *Aphis rumicis* and compared with recently prepared samples containing 10 to 20 per cent. of the ammoniated emulsifier. The data are set out in Table VI.

The data demonstrate that the loss of toxicity in these preparations at either temperature has not been any greater than the loss in the same period in the concentrated extract of pyrethrum in petroleum-ether (4.5 per cent. pyrethrin I) from which they were made. There may have been some loss of toxicity of questionable significance at 28° C. in the case of the sample containing 40 per cent. of the emulsifier, an amount hardly likely to be used in practice.

A series of tests with lubricating oil and pyrethrum were incorporated to ascertain the stability of the poisons and the degree to which the toxicity was increased by the heavy oil. We do not suggest that the mixture experimented with is the most suitable that could be made; it could, however, be emulsified with rather vigorous stirring with both soap and saponin solutions. In some field trials against a species of capsid bug (*Lygus pabulinus* Linn.) on red currants, the lubricating oil-pyrethrum mixture when emulsified with soap gave good results, practically complete control being established at low concentrations. The following was the method of preparation. A highly concentrated petroleum-ether extract of pyrethrum flowers was prepared for us and further concentrated to a rather viscous fluid. The volatile acid after saponification was determined in the way outlined by us and the amount found calculated to pyrethrin I(10). It gave a figure of 4.5 gm. per 100 c.c. The use of so concentrated an extract allowed highly diluted emulsions to be made, and thus the amount of lubricating oil used for spraying could be cut down to concentrations below that likely to kill the insects when used by itself in control tests, or do damage to foliage. 10 gm. of Agral W.B. to which had been added 0.5 c.c. of strong ammonia (s.g. 0.9) were incorporated with about 85 c.c. of the lubricating oil and 11.1 c.c. of the strong pyrethrum extract. 4 c.c. of oleic acid were added to correct a slight turbidity noticed on cooling to 0° C. With different proportions of ammonia or other heavy oils the amount of oleic acid added would need modification, but it should be cut down to a minimum required to clear. The lubricating oil used was known as refined 50 grade. The mixture was divided into two portions, one of which was kept at laboratory temperature and the other at 28 to 30° C. for 5 months. Tests

were carried out in the usual way by preparing dilutions with 0.5 per cent. saponin—the concentration used being 0.001, 0.005 and 0.00025 gm. per 100 c.c. in terms of pyrethrin I. In addition, tests were made with a sample prepared in the same way the day before spraying and with the highly concentrated stock extract of pyrethrum used in its preparation.

Table VII. *Toxicities to A. rumicis of mixtures of pyrethrum extracts and lubricating oil.*

N. = not affected; S. = slightly affected; M. = moribund; D = apparently dead.

Results 3 days after spraying.

Preparation	Concentration pyrethrin I as determined from volatile acid	N. %	S. %	M. %	D. %	M. and D. %
*Pyrethrum-lubricating oil mixture containing 10 % ammoniated Agral W.B. ammonia = 0.05. Stood at lab. temp. 5 months	0.001	—	—	10	90	100
	0.0005	20	—	10	70	80
	0.0025	60	—	20	20	20
Ditto. Stood at 28–30° C. 5 months	0.001	—	—	10	90	100
	0.0005	30	10	20	40	60
	0.0025	70	10	—	20	20
Ditto. Freshly prepared	0.001	—	—	20	80	100
	0.0005	10	20	20	50	70
	0.0025	40	20	10	30	40
Agral W.B. and all solvents as used in highest concentration	—	100	—	—	—	—
0.5 % solution of saponin	—	100	—	—	—	—
Strong extract of pyrethrum in low boiling petroleum-ether used in above mixtures (no lubricating oil or Agral W.B. present)	0.002	—	—	20	80	100
	0.001	—	40	40	20	60
	0.0005	70	10	—	20	20

\* On cooling to 0–3° C. for 24 hours this mixture remained clear.

The control tests with 0.5 per cent. saponin solution and the lubricating oil and ammoniated Agral W.B. gave completely negative results. It will, however, be observed that the pyrethrum extracts containing lubricating oil and Agral W.B. are significantly more toxic than the petroleum-ether extract from which they were prepared. It is thus clear that these higher fractions of petroleum enhance the toxicity of pyrethrum, even when the petroleum is present in sub-lethal concentrations. There is little difference in toxicity between the three pyrethrum-lubricating-oil tests and any discrepancies observed cannot be regarded

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as outside experimental error. Pyrethrum would therefore appear to be relatively stable in such extracts as the above, particularly in temperate climates.

*Turkey-red oil series.* Sulphonated castor oil has been known for some considerable time as an emulsifier, and neutralised material has been suggested by Harder(2) for use with benzene, petroleum-ether, and trichlorethylene extracts of pyrethrum. Turkey-red oil is more soluble in water than neutral Agral W.B., but it is less soluble in organic solvents, therefore miscible oils prepared with it require most careful balancing of solvent to oil if the mixture is to remain clear. Mixtures prepared from turkey-red oil, however, pass into water much more readily than those in which Agral W.B. is used, and have the advantage, as is shown below, of mixing well with magnesium-hard water. Separation may in some cases be comparatively rapid, but gentle stirring of the spray mixture in the tank will keep the emulsion sufficiently uniform for spraying purposes. Although mixtures prepared from turkey-red oil can be used without a wetter they would appear to require its addition in the spray tank in order to secure maximum efficiency. Commercial turkey-red oil is usually alkaline in reaction; it is therefore advisable to neutralise before using it in the presence of the pyrethrins. Harder(2) suggests for this purpose the addition of a little 50 per cent. acetic acid, but it should be possible for neutrality to be secured by the manufacturer by a suitable washing process. One sample used by us was neutral but contained much wash liquor which gradually settled. The sample was therefore allowed to stand for some time and the clear oil carefully decanted off. Another sample was distinctly alkaline in reaction and before use was mixed with an equal volume of refined oleic acid—the product was then neutral to litmus. The addition of oleic acid enables a larger amount of petroleum spirit to be incorporated, but it introduces the disadvantage that the mixture now no longer disperses freely in water, a difficulty, however, that can be overcome by rendering the latter slightly alkaline by the addition of a little sodium carbonate. The turkey-red oil—oleic acid mixture, moreover cannot be used with magnesium-hard water, clotting and reversal of phase taking place. The petroleum fraction used was semi-refined white spirit with the following characteristics:

Specific gravity at 60° F.	...	...	0.782
Flash point (Abel)	...	...	94° F.
About 78 per cent. distilled below	...	...	175° C.

It contained a certain portion of aromatic hydrocarbons. Three preparations of the following composition were tested:

- |   |           |
|---|-----------|
| A. <sup>1</sup> Concentrated pyrethrum extract in petroleum-ether   | 11.1 c.c. |
| Semi-refined white spirit     ...     ...     ...     ...   | 18.9 c.c. |
| Neutral turkey-red oil to bring to a volume of     ...  | 102 c.c.  |
|   |           |
| B. <sup>1</sup> As A, but 18.9 c.c. of a mixture of tetrachlorethane 40 c.c. and semi-refined white spirit 60 c.c. were used. (This gives a higher specific gravity and so prevents the oil rising quickly to the surface.) |           |
|   |           |
| C. Concentrated pyrethrum extract in petroleum-ether  | 11.1 c.c. |
| Alkaline turkey-red oil     ...     ...     ...     ...   | 16.6 c.c. |
| Oleic acid     ...     ...     ...     ...     ...  | 16.6 c.c. |
| Semi-refined white spirit to a volume of     ...     ...  | 100 c.c.  |

These mixtures were divided into two portions, one being kept at laboratory temperature, the other at 28 to 30° C. After periods of time given in Table VIII A and VIII B each was diluted with a solution of 0.5 per cent. saponin in water and tested against a recently prepared sample. The data are given in Tables VIII A and VIII B.

Inspection of Table VIII A and VIII B indicates that the control tests with turkey-red oil and the solvents alone have lethal properties, particularly where tetrachlorethane has been used. Some of this effect is probably mechanical, due to the turkey-red oil, but in addition some toxicity would be expected from tetrachlorethane. Although on this day no trials were possible with the concentrated pyrethrum extract used in the preparation of these mixtures, it is safe to say the turkey-red oil and solvents have enhanced the toxicity of pyrethrum, and although the samples A and B which have stood at the temperature of the laboratory show no loss of toxic properties as compared with the freshly prepared sample, both the samples kept at 28° C. are probably significantly lower in toxicity. Preparations of the type A and B would apparently retain their toxicity in temperate climates over a sufficiently long period.

In addition to the tests after a period of 5 months a further series was carried out with mixture A after 15 months, the results of which are set out in Table VIII B. These tend to confirm the deduction drawn from the data in Table VIII A. It is, however, surprising that after so

<sup>1</sup> A and B separated out a thin layer on surface on standing. The amount of petroleum present has not been adjusted sufficiently exactly.

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long a period alkali-free turkey-red oil has so little detrimental action upon the toxicity of the pyrethrins.

Table VIII A. *Permanence of toxicity of pyrethrum turkey-red oil preparations.*

N. = not affected; S. = slightly affected; M. = moribund; D. = apparently dead.

Results 3 days after spraying.

Preparation		Concen- tration pyrethrin I as deter- mined from volatile acid	N. %	S. %	M. %	M. and D.	
						D. %	D. %
Mixture A (see p. 221). Stood at lab. temp. 4 months 29 days	(1)	0.002	—	—	—	100	100
	(2)	0.001	—	—	—	100	100
	(3)	0.0005	—	10	20	70	90
Mixture A. Stood at 28–30° C. 4 months 29 days		0.002	—	—	20	80	100
		0.001	—	—	30	70	100
		0.0005	60	10	—	30	30
Mixture A (freshly prepared)		0.002	—	—	—	100	100
		0.001	—	—	10	90	100
		0.0005	—	15	25	60	85
Control with turkey-red oil and all sol- vents used in	(1)	—	90	—	—	10	10
	(2)	—	80	—	10	10	20
	(3)	—	100	—	—	—	—
Mixture B (see p. 221). Stood at lab. temp. 4 months 29 days	(4)	0.002	—	—	—	100	100
	(5)	0.001	—	—	—	100	100
	(6)	0.0005	10	10	40	40	80
Mixture B. Stood at 28–30° C. 4 months 29 days		0.002	—	—	—	100	100
		0.001	—	10	50	40	90
		0.0005	30	30	—	40	40
Control with turkey-red oil and all sol- vents as in	(4)	—	40	10	—	—	—
	(5)	—	100	—	—	—	—
	(6)	—	80	—	10*	10*	20
0.5 % solution of saponin		—	100	—	—	—	—

\* These insects were of poor quality.

Both mixtures A and B became slightly turbid on cooling to 0–3° C. for 24 hours. On standing at low temperature they separated out a thin layer on surface; adjustment of components had not been exact enough.

Table VIII B, contains data concerning the permanence of toxicity of mixture C, in which the alkalinity of a commercial sample of turkey-red oil was more than neutralised by the addition of oleic acid. Oleic acid and its soap are known to have a lethal effect upon *Aphis rumicis*; this, however, was not pronounced in the controls at the concentrations tested, although it would appear evident that the toxicity of the pyrethrins has been enhanced by its presence. The data show that there has been little or no loss of toxicity of the mixture after standing 6–7 months either at the laboratory temperature or at 28 to 30° C.



Table VIII B. *Permanence of toxicity of pyrethrum turkey-red oil preparations.*

N. = not affected; S. = slightly affected; M. = moribund; D. = apparently dead.

Results 3 days after spraying.

Preparation	Concen- tration pyrethrin I %	N. %	S. %	M. %	D. %	M. and D. %
<i>Series A:</i>						
Mixture A (see p. 221). Stood at lab. temp. 15 months	(1) 0.002	—	—	—	100	100
	(2) 0.001	10	—	10	80	90
	(3) 0.0005	60	10	10	20	30
Mixture A. Stood at 28–30° C. 15 months	0.002	—	—	10	90	100
	0.001	20	—	—	80	80
	0.0005	90	10	—	—	—
Mixture A (freshly prepared)	0.002	—	—	—	100	100
	0.001	—	—	30	70	100
	0.0005	20	—	40	40	80
Control solvents etc. as	(1) —	90	—	10	—	10
	(2) —	90	10	—	—	—
	(3) —	90	—	—	10	10
<i>Series B:</i>						
*Mixture C (see p. 221). Stood at lab. temp. 6–7 months	(3) 0.001	—	—	—	100	100
	(4) 0.0005	10	5	20	65	85
	(5) 0.00025	40	5	10	45	75
Mixture C. Stood at 28° C. 6–7 months	0.001	—	—	—	100	100
	0.0005	10	10	15	65	80
	0.00025	25	—	15	60	75
Mixture C (recently prepared)	0.001	—	—	10	90	100
	0.0005	—	—	5	95	100
	0.00025	40	—	10	50	60
Control solvents etc. as	(3) —	95	—	—	5	5
	(4) —	85	—	—	15	15
	(5) —	85	—	5	10	15

\* Mixture C shows only a slight deposit on cooling to 1° C.

Mixture C goes into water far less readily than either A or B, and, as will be shown later, requires for stable emulsification an addition of alkali to the dispersion medium. It can then be used with calcium-hard waters; magnesium-hard waters, however, cause a reversal of phase to the water-in-oil state, in marked contrast to their effect on the mixtures A and B.

*Permanence of poisons in dilute emulsions.* It will be shown later that not only does the presence of a weakly dissociated alkali in the miscible oils prepared from Agral W.B. aid the readiness of emulsification and stability of the emulsion formed, but for both of these purposes a certain degree of alkalinity in the water of the spraying tank is desirable. It was, therefore, considered important to ascertain how long poisons of pyrethrum would remain active when dispersed in water of

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different degrees of alkalinity. Accordingly, an extract of pyrethrum was added to solutions of 0.5 per cent. saponin buffered at pH 7, 9 and 11. The concentration in terms of pyrethrin I, as calculated from the volatile acid, was 0.002 gm. per 100 c.c. After standing, this emulsion was tested, as were also dilutions of 0.001 and 0.0005 gm. per 100 c.c. Tests were made with samples which were prepared just before spraying and with others that had remained in contact with the buffered dispersions medium for 27 hours and 25 days. One series of tests was carried out with a sample which had stood for 25 days in the presence of *N/10* soda. Unfortunately, it was found that the buffer used had a considerable but delayed toxic action, and therefore the numerical data were of little value. It was, however, noticed that all the buffered mixtures ranging from pH 7 to pH 11 showed the almost instantaneous narcosis characteristic of the physiological action of pyrethrum. The preparations made with *N/10* soda showed a loss of toxicity after 25 days.

Table IX. *Stability of the active principles of pyrethrum when dispersed in alkaline solutions.*

N. = not affected; S. = slightly affected; M. = moribund; D. = apparently dead.

Results 2 and 3 days after spraying.

Condition of experiment	Concentration pyrethrin I as determined from volatile acid	After 2 days						After 3 days					
		N.	S.	M.	D.	M. and D.		N.	S.	M.	D.	M. and	
		%	%	%	%	%	%	%	%	%	%	%	%
<i>N/10</i> soda unbuffered.	(1) 0.002	—	—	—	100	100	—	—	—	100	—	100	—
Stood at lab. temp.	(2) 0.001	10	20	40	30	70	50	—	10	40	50	—	—
5 days	(3) 0.0005	90	—	—	10	10	80	—	—	20	20	—	—
<i>N/10</i> soda unbuffered.	0.002	—	—	—	100	100	—	—	10	90	100	—	—
Stood at lab. temp.	0.001	—	20	30	50	80	30	20	—	50	50	—	—
about 24 hours	0.0005	50	20	20	10	30	50	20	—	30	30	—	—
<i>N/10</i> soda unbuffered.	0.002	—	—	—	100	100	—	—	—	100	100	—	—
Prepared just before spraying	0.001	—	10	10	80	90	—	10	10	80	90	—	—
	0.0005	—	10	40	50	90	50	20	20	10	30	—	—
Control as No. 1 but no pyrethrum present	—	70	—	20	10	30	70	—	10	20	30	—	—
Control as No. 2 but no pyrethrum present	—	100	—	—	—	—	80	—	—	20	20	—	—
Control as No. 3 but no pyrethrum present	—	100	—	—	—	—	70	—	—	30	30	—	—
pH 11 buffered by glycine. Stood at lab. temp. 5 days	0.002	—	—	—	100	100	—	—	—	100	100	—	—
	0.001	—	—	50	50	100	—	10	20	70	90	—	—
	0.0005	20	—	30	50	80	30	—	40	30	70*	—	—
pH 11 buffered by glycine. Stood at lab. temp. 24 hours	0.002	—	—	—	100	100	—	—	—	100	100	—	—
	0.001	—	—	20	80	100	—	10	50	40	90	—	—
	0.0005	10	30	30	30	60	50	20	—	30	30	—	—
pH 11 buffered by glycine. Prepared just before spraying	0.002	—	—	—	100	100	—	—	—	100	100	—	—
	0.001	—	—	—	100	100	—	10	10	90	100	—	—
	0.0005	—	—	30	70	100	10	10	—	80	80	—	—

\* This value probably slightly exaggerates toxicity; there were a large number of moribund.

A fresh series of experiments were set up with the dispersion medium at pH 11, Sorensen's glycine caustic soda mixture being used as buffer, and with *N*/10 caustic soda. The insects had to be evaluated on the second day after spraying, as the control tests showed losses after that period (the third day's results are also given in Table IX); also it should be noted that the *N*/10 soda produced a number of deaths, probably due to its irritant action.

The data in Table IX do not allow of strictly quantitative comparisons owing to the poor quality of the control tests. It may be safely concluded, however, that after 5 days and even after 24 hours in the presence of *N*/10 soda there is with pyrethrum some loss of toxicity; the fact that there is so little difference between the results obtained after 24 hours and those after 5 days is probably due to the partial neutralisation of the soda by resin acids present in the extracts. There is some indication also of a slight loss when the alkalinity is reduced to pH 11. It is, however, a matter of surprise that the loss of toxicity is so small at these degrees of alkalinity and as such highly caustic spray fluids as that given in this case by *N*/10 soda are not likely to occur in practice, it would appear that pyrethrum extracts would show a relatively small decline under normal circumstances after remaining in the spray tank for a few days. As, however, loss of toxicity is likely to take place progressively the diluted spray mixture should not be left too long in the tank.

#### EFFECT OF COOLING ON WATER-MISCIBLE OILS.

It is important that miscible oils should remain comparatively clear on cooling to temperatures of about 0° C. This problem has been studied by Hart(3) who, for a variety of such oils, traces out the factors necessary for clarity to be maintained at low temperatures, and drew for acid sulphonated castor oil mixtures the following conclusions: (1) in the absence of alcohol the addition of alkali at first decreases and then increases the amount of free oleic acid required for a homogenous product; (2) if alcohol is present in sufficient quantity the more alkali present less oleic acid is required to clear the oil; (3) the more neutralised the sulphonated oil the better it functions as emulsifier, the completely neutralised oil being best in this respect. Oleic acid in proper balance often acts as a clearing agent in miscible oils. Hart's paper is of importance to those interested in the preparation of these oils.

The toxicity data presented in Tables VIII A and VIII B in connection with neutral sulphonated castor oil (turkey-red oil) were for

rather extreme conditions, so as to obtain maximum effects on toxicity and as no oleic acid was added only a minimum of light petroleum oil could be used; but it should be pointed out that the conditions under which these oils may be used, for example in calcium and magnesium-hard water, demands that for clearing purposes a minimum amount of oleic acid should be used in order to avoid clotting and possible reversion. Except in one case (mixture C, p. 221) the use of a considerable proportion of free oleic acid has been avoided in our mixtures.

All our miscible oil preparations were subjected to cooling to temperatures of  $0^{\circ}$ – $3^{\circ}$  C. in an "Electrolux" refrigerator for 24 hours, the effect of cooling being indicated in the tables. All oils of this type on being subjected to cooling to  $0^{\circ}$  C. should remain fairly clear, otherwise, there is risk, through the formation of two phases or the separation of some of the constituents, of the effectiveness being lost. A trace of turbidity is hardly likely to be of importance unless it leads to extensive phase separation. In our tests the only sample to show extensive separation was one containing oleic acid saturated with dry ammonia gas, and it is highly probable that a change in proportion of alcohol to petroleum-ether would have acted as a correction. The turkey-red oil samples A and B showed some turbidity, and it is probable that the ideal combination of petroleum and sulphonated oil was not realised and certain of the Agral-ammonia samples deposited a trace of precipitate, but with pyrethrum extracts such precipitates are probably traces of resin acids and they are not likely to be of importance.

#### READINESS OF EMULSIFICATION OF PYRETHRUM EXTRACTS AND STABILITY OF EMULSION.

For the purpose of investigating the emulsibility of pyrethrum extracts and the stability of the resulting emulsions, we have used as far as possible solutions in which the stability of the poisons had been tested as already described.

In a separate publication one of us (R.P.H. (4)) carried out an investigation of the interfacial tension of pyrethrum extracts against aqueous solutions, with particular reference to the concentration of hydrogen and calcium-ion; a matter of importance as hard waters may be used in making up spray fluids. While a low interfacial tension is conducive to spontaneous emulsification it does not necessarily imply that the resulting emulsion is stable, and it therefore appeared necessary to make up emulsions under the conditions suggested by the study of interfacial tensions and to test their actual stability. The oil content of emulsions

left to stand various periods was estimated by a technique which consisted essentially in breaking the emulsion with acid, the oil being separated and its volume measured by the Gerber method for the estimation of fat in milk.

#### EMULSIFICATION OF PYRETHRUM EXTRACTS IN HARD WATERS.

Harpenden tap-water was used in these experiments. It was found by analysis to contain 27.6° temporary hardness and 30° total hardness only traces of magnesium were present. The temporary hardness was in addition determined in samples taken over several days by the soap method. On only one occasion was it found to fall below 26°. For testing out higher concentrations of calcium and the effects of magnesium salts calcium and magnesium sulphates were added respectively.

*Preliminary test-tube experiments.* Solutions were made up in tap-water as follows:

1.	0.1	per cent.	$\text{Na}_2\text{CO}_3$	
2.	0.1	„	„	0.5 per cent. Agral I <sup>1</sup>
3.	0.075	„	„	„ „
4.	0.05	„	„	„ „
5.	0.025	„	„	„ „

0.5 c.c. of the various miscible oils dealt with above were added to 10 c.c. of the aqueous solution and, after shaking, the mixture was left to stand. All the mixtures made with the pyrethrum extract solution without Agral W.B. broke in a few minutes; with the pyrethrum extract solution containing 10 gm. per 100 c.c. of Agral W.B. the emulsions broke rapidly except at the highest concentration of sodium carbonate, 0.1 per cent.; this was the concentration at which Hobson (*loc. cit.*) found the interfacial tension became immeasurable. In this case two of the factors for stability are (1) very low interfacial tension, (2) the presence of a stabilising agent. It is possible that the necessity of adding an emulsifying agent might be avoided by the use of a higher concentration of sodium carbonate, but this was not tested, as a minimum of alkalinity was desirable.

To test out the effect of the magnesium and of calcium in larger amounts, solutions of the sulphates were made up in tap-water. A solution containing pyrethrum extract and 10 gm. per 100 c.c. of Agral W.B.

<sup>1</sup> Agral I is a proprietary article which lowers the surface tension of water and so aids wetting.

was used and the stability of the emulsions observed. With only calcium present it was found that stable emulsions could be formed provided sufficient sodium carbonate or sodium phosphate were added; the precipitate did not settle out or in any way interfere with the emulsion. With solutions of magnesium salts containing various amounts of sodium carbonate, phosphate or hydroxide no stable oil-in-water emulsions resulted; either oil or the water-in-oil phase separated. The failure in the case of sodium carbonate was probably due to the relatively high solubility of magnesium carbonate; the ratio  $[Mg^{++}]/[OH']$  is probably important, as the ratio  $[Ca^{++}]/[OH']$  was found by Hobson (*loc. cit.*) to influence the interfacial tension. When sodium phosphate or hydroxide or ammonia was added, the magnesium was precipitated and quickly formed a curd which rose to the surface and broke the emulsion. A few experiments were made using casein and gelatine as emulsifiers where magnesium was present; the results, although better, were not sufficiently encouraging to justify continuation of the experiments.

#### QUANTITATIVE DETERMINATION OF THE RELATIVE STABILITY OF THE EMULSIONS.

The preliminary experiments indicated that no grave difficulties arise with water containing calcium only, but that the problem with magnesium present is far more serious. To obtain further and more exact data a quantitative technique was worked out.

*Method.* The emulsions were prepared and poured into a 400 c.c. pear-shaped separating funnel, identical funnels being used throughout. At intervals 25 c.c. were run out slowly and 20 c.c. taken for determination of the oil content. Although a gradient in the oil concentration with depth probably existed, the small proportion of the total volume taken would minimise any error, which would also fall evenly and not affect the comparative accuracy of the results.

The amount of oil remaining in suspension was determined by demulsifying and measuring the volume of the oil. The apparatus and principles of the Gerber method of estimating butter-fat in milk was employed. It may be noted that Griffen and Richardson<sup>(1)</sup> adapted the Babcock method of determining butter-fat in milk for a similar use with oil-sprays. Our procedure was as follows: 20 c.c. of the emulsion were placed in a Gerber butyrometer tube and 2 c.c. of the acid mixture were added. The acid mixture contained 10 per cent. hydrochloric acid and 10 per cent. sodium chloride and 1 per cent. ferric chloride. The excess of

carbon dioxide was shaken out and the rubber bungs pressed in tightly, by whirling in a centrifuge most of the oil was separated; after standing overnight and centrifuging again, the oil was completely separated, the water layer being crystal clear. Occasionally it was necessary to allow the tubes to stand for a further few hours. The volume of oil was read off in the units on the scale, which correspond to 1 per cent. fat in 11 c.c. of milk and which have been calculated to be approximately 0.125 c.c. in volume. Thus if the density of butter-fat at 70° (at which readings of butter-fat are made) is taken as 0.88, then the volume of 1 unit ( $v$ ) is as follows:

$$\frac{v \times 0.88}{11} = \frac{1}{100},$$

$$v = 0.125 \text{ c.c.}$$

This value was confirmed by determination with freshly shaken emulsions of known strength.

In most of the experiments a solution of 5 per cent. pyrethrum extract in semi-refined white spirit was emulsified in Harpenden tap-water containing 0.1 per cent. sodium carbonate and 0.5 per cent. Agral I, various substances being added to each phase. The concentration of oil at the start was 2 per cent., except in a few cases when it was 1 per cent. This amount is rather higher than would in general be employed for spraying, but allows more accurate reading on the Gerber tubes. In our experiments there is probably a certain experimental error introduced by the absorption of water by the oil film, but on the whole the results are comparable with each other.

*Results.* The results are contained in Table X. The main points examined were the influence of the method of making up the emulsion, of the presence of the ammonia and the concentration of Agral W.B. in the pyrethrum solution, and of the presence of varying amounts of calcium and magnesium in the aqueous phase. The results will be discussed in this section under these main heads and in addition a few experiments with turkey-red oil solution will be described. Analyses were carried out with most of the mixtures after intervals of 1, 6 and 24 hours and the general type of curve obtained is shown in Diagram 1, in which the percentage of oil remaining in suspension is plotted against time. An apparent equilibrium is reached in 24 hours, after which the separation of oil becomes immeasurably slow. The rate at which this equilibrium is reached is nearly the same in all cases, and examination of Table X shows that it is immaterial whether comparisons are made on the basis of the results after 1, 6 or 24 hours.

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Table X. *Stability of emulsions prepared from water-miscible petroleum spirit extracts of pyrethrum.*

Series A:	Oil phase. Pyrethrum solution containing		Method of pre- paration	Per- centage oil in emul- sion	Aqueous phase	Percentage of the oil added found in emulsion after			
	Ammonia % (vol.)	Agral W.B. %				½ hr.	1 hr.	6 hrs.	24 hrs.
1	0	10	A	2	Tap-water containing 30° of hardness as calcium, 0.5 % Agral I and 0.1 % sodium car- bonate being added	<3	—	—	—
2	0	10	A*	2		20	—	—	—
3	0	10	B	2		6	—	—	—
4	0	10	C	2		58	—	—	—
5	0	10	C	1		81	—	—	—
6	0	10	C	1		81	62	45	44
7	0	10	C	2		64	48	34	31
8	(Solvent)	10	C	2		—	63	42	30
9	1	10	A	2	Ditto + 10° Mg " + 20° " " + 50° " " + 100° " " 100° Mg } 0.1 % NaOH } 0.3 % Na <sub>2</sub> CO <sub>3</sub> } 0.1 % Na <sub>2</sub> CO <sub>3</sub> } 0.2 % Na <sub>2</sub> CO <sub>3</sub> } 0.1 % Na <sub>2</sub> CO <sub>3</sub> } 0.1 % amm. oxalate " 100° Ca } 0.1 % Na <sub>2</sub> CO <sub>3</sub> } 0.2 % Na <sub>2</sub> CO <sub>3</sub> Agral I } Agral I } Agral I }	—	89	67	47
10	2	10	A	2		—	72	42	36
11	2	10	C	2		—	48	38	33
12	2	10	B	2		—	44	30	27
13	4	20	B	2		—	80	66	52
14	4	20	A	2		—	85	72	60
15	2	20	A	2		—	89	75	64
16	2	40	A	2		—	76	66	60
17	5	40	A	2		—	95	93	91
18	3.5	25	A	2		—	83	68	61
19	3.5	30	A	2		—	92	81	77
20	2	16	A	2		—	81	—	—
21	5	40	A	2		—	80	—	—
22	5	40	A	2		—	72	—	—
23	5	40	A	2		—	42	—	—
24	5	40	A	2		—	20	—	—
25	5	40	A	2		—	59	—	—
26	5	40	A	2		—	30	—	—
27	2	20	A	2		—	3	—	—
28	2	20	A	2		—	5	—	—
29	2	20	A	2		—	8	—	—
30	5	40	A	2		—	75	70	—
31	5	40	A	2		—	94	91	—
32	2	20	A	2	Tapwater + 0.5 % soft soap	—	<5	—	—
33	2	20	A	2	Ditto; + 0.1 % Na <sub>2</sub> CO <sub>3</sub>	—	98	86	72
Series B:						Tapwater; 30° Ca			
34	Mixture A	Turkey-red	A	2	+ 0.5 % Agral I	—	64	—	—
35	"	oil	A	2	+ 0.5 % Agral I, 0.05 % Na <sub>2</sub> CO <sub>3</sub>	—	69	—	—
36	"	70/100 c.c.	A	2	+ 0.5 % Agral I, 0.1 % Na <sub>2</sub> CO <sub>3</sub>	—	66	—	—
37	"		B	2	+ 0.5 % Agral I	—	80	—	—
38	Mixture B		B	2	+ 0.5 % Agral I	—	44	—	—
39	"		B	2	+ 0.5 % Agral I, 100° Mg	—	44	—	—
40	Mixture A		B	2	+ 0.5 % Agral I, 100° Mg	—	28	—	—

\* Intermittent shaking for 50 minutes.

*Method of preparing emulsion, and the effect of the presence of ammonia in oil.* The aqueous solutions were prepared by diluting a stock solution of sodium carbonate and Agral I (in tap-water) ten times with tap-water, the final concentrations being 0.1 and 0.5 respectively. The emulsions were made up in the following three ways:

*Method A.* Stock solutions\* diluted, oil poured into the whole of solution, mixture shaken.



*Method B.* As A, except that oil was emulsified in 10 per cent. of solution by shaking, then poured into the remaining 90 per cent.

*Method C.* Oil emulsified in mixtures of the ten times concentrated stock solution, then diluted with tap-water. The period of shaking was from 1 to 3 minutes unless otherwise specified.

With the pyrethrum solution containing 10 per cent. Agral W.B. but no ammonia, method C gave a fairly stable 2 per cent. oil emulsion which contained 64 per cent. of the added oil after 15 minutes, but the emulsions prepared by methods A and B broke almost immediately (Table X, nos. 1-7). A comparison of tests 1 and 2 shows that shaking for a longer time with intervals of rest gave more stable emulsions. The

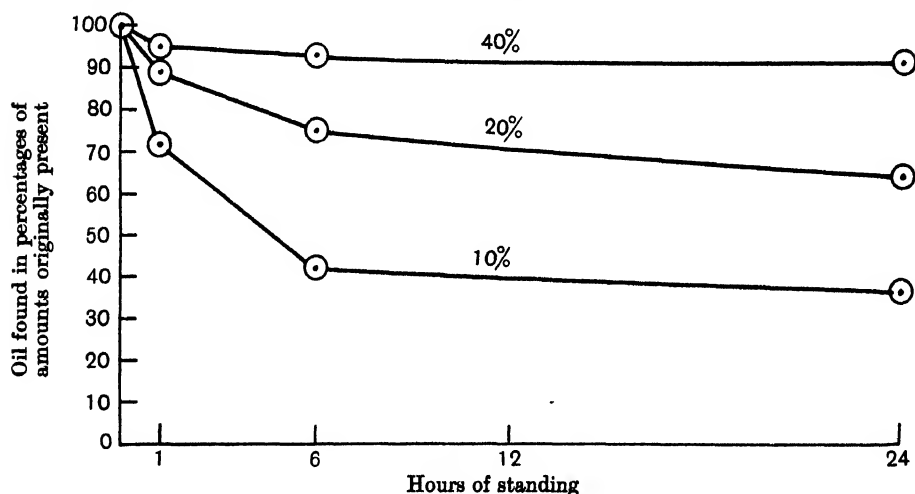


Diagram 1. Stability of emulsions—effect of time of standing. The emulsions contained originally 2 % petroleum spirit extract of pyrethrum and varying proportions of Agral W.B. (ammoniated).

question of shaking was not investigated further, as it was found that addition of small amounts of ammonia to the pyrethrum-Agral solutions facilitated emulsification.

With a pyrethrum solution containing 10 per cent. Agral and 2 per cent. (by volume) of ammonia (s.g. 0.9), method A gave the most stable emulsion. Taking as a basis of comparison the percentage of the added oil found in suspension after 1 hour, the presence of the ammonia is seen to raise the figure from 3 per cent. to 72 per cent. for method A and from 6 per cent. to 44 per cent. for method B, but to leave it unchanged for method C (Table X, nos. 10-12). We can offer no explanation of the fact that methods B and C give less stable emulsions with ammoniated pyrethrum solutions than does method A.

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The action of the ammonia is probably as follows. On mixing with water the ammonia in the oil will pass into the water and produce a high alkalinity at the interface for a very short time. It has been shown by Hobson (*loc. cit.*) that the interfacial tension with pyrethrum solutions in petroleum fall with rise in *pH*; with the actual solutions used in these tests the conditions were such that the interfacial tension is immeasurably small. It is not impossible, however, that by further increasing the alkalinity of the aqueous phase the tension may continue to decrease; that is the curve relating the tension to the *pH* becomes asymptotic as

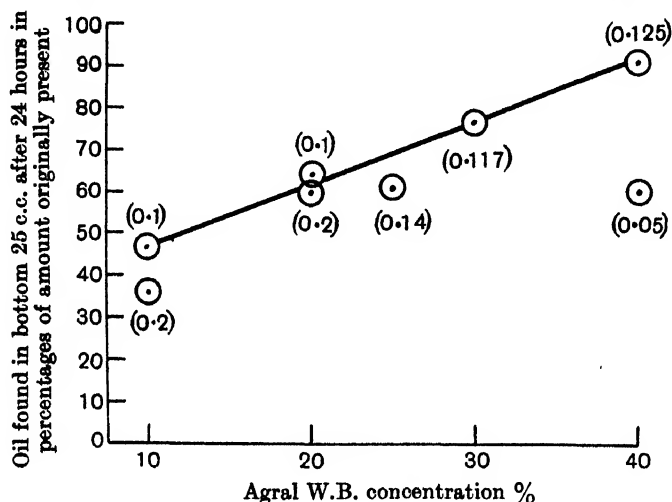


Diagram 2. Relation between Agral W.B. and ammonia contents of petroleum spirit extracts of pyrethrum and the stability of their emulsions.

Dispersion medium—tap water of 30° Ca hardness containing 0.5 % Agral I and 0.1 %  $\text{Na}_2\text{CO}_3$ .

Figures in brackets indicate ratio  $\frac{0.9 \text{ ammonia c.c.}}{\text{Agral W.B. gm.}}$ .

the tension approaches zero. If this be so, it can be readily understood how the ammonia assists the oil to emulsify in the first place, without changing significantly the stability of the resulting emulsion, as the final concentration of the ammonia is very low.

*Effect of ammonia and Agral W.B. concentration on the stability of the emulsion.* The results given in Table X, nos. 9–20, show that increasing the concentration of Agral W.B. in the oil improves the stability of the resulting emulsion increases, provided the ratio of ammonia to Agral W.B. lies within certain limits. The data are insufficient to determine whether there is an actual optimum ratio, but this conclusion might be drawn from Diagram 2, in which the percentage of oil remaining in

emulsion after 24 hours is plotted against the percentage of Agral W.B. in the pyrethrum solution, the numbers in brackets by each point representing the ratio ammonia c.c./Agral W.B. gm. This diagram shows that the points which correspond to ratios between the limits of 0.1 and 0.125 lie along a straight line, the values for ratios outside these limits falling below the line. Where the ratio is less than 0.1, the amount of available ammonia is probably insufficient to disperse the oil at the moment of emulsification. At ratios higher than 0.125 there is some evidence of the stability falling off; this may be a viscosity effect, for as the ratio of ammonia to Agral W.B. increases, the viscosity becomes higher and the initial mixing of oil and water more difficult. Violent shaking effects this in laboratory tests but under field conditions it might prove difficult to mix certain of these more viscous solutions with water.

*Gradient of separation of emulsions.* To obtain further information on the separation of these mixtures, we decided to do a more elaborate series of tests and determine the proportion of oil found at different levels after the emulsions, prepared from water-miscible extracts, had stood 24 hours. The pyrethrum-miscible oils contained varying amounts of ammoniated Agral W.B., the ratio of ammonia to Agral W.B. being kept at 0.1. The data given in Table X are for very drastic conditions—the bottom levels from a conical funnel being taken off and the oil determined. This procedure not only gave no information as to how the concentration of the oil varied with depth, but actual experiment indicated that the shape of the funnel expedited separation. It was, therefore, decided to carry out certain experiments in a cylindrical vessel.

A straight tube of even bore throughout, every 5 cm. of which corresponded to 25 c.c. volume, was fitted with a rubber stopper holding a glass-tap. The rubber stopper and tube above the tap was covered with mercury, to prevent the emulsion coming into contact with the rubber and from running in the narrower tube of the stop-cock. Water-miscible pyrethrum extracts containing 5, 10, 20 and 40 per cent. of ammoniated Agral W.B., ratio  $\frac{\text{ammonia (0.9)}}{\text{Agral W.B.}} = 0.1$ , were prepared, and 2 per cent. oil emulsions were made by pouring each into a cylinder containing a measured quantity of a solution of 0.5 per cent. Agral I and 0.1 per cent. sodium carbonate in tap-water (Ca hardness 30°). Each was shaken for half a minute and poured into the separation tube. After standing 24 hours, the mercury above the upper tube of the stop-cock was gently

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run out and each 25 c.c. from the bottom of the emulsion to the level of the cream very gently run off. These were shaken and 20 c.c. used for the determination of the oil by the method already described. Although no great accuracy is claimed for this method, the fractions run off, approximate in composition to the successive layers in the tube, provided the flow is sufficiently slow. (The gradations in turbidity were noticed even in the cylinders into which the emulsions were run.) There is some difficulty in shaking in exactly the same way in every case, but we believe the results on the whole to be comparable with each other.

Table XI. *Degree of separation of pyrethrum petroleum spirit emulsions containing ammoniated Agral W.B.*

Ratio $\frac{0.9 \text{ ammonia c.c.}}{\text{Agral W.B. gm.}} = 0.1.$				
Dispersion medium: Ca-hard water 30° total hardness containing 0.5 % Agral I and 0.1 % $\text{Na}_2\text{CO}_3$ .				
Mean amount of oil found after 24 hours in each 5 cm. of height in percentages of original amount present				
Height in cm.	5 % ammoniated Agral W.B.	10 % ammoniated Agral W.B.	20 % ammoniated Agral W.B.	40 % ammoniated Agral W.B.
0-5	10	51	89	96.1
5-10	22.7	73.4	97.5	97.7
10-15	46.1	81.3	103.1	97.7
15-20	60.9	84.4	103.1	98.4
20-25	74.2	87.5	104.7	98.4
25-30	78.1	88.4	105.5	98.4
30-35	82.8	89.0	104.7	—
35-40	89.0	92.2	105.5	98.4
40-45	90.6	95.3	105.5	99.2
45-50	94.5	96	105.5	99.2
50-55	94.5	96	105.5	99.2
55-60	Much cream— separated clear oil	Some cream	A little cream	103

*Note.* Each water-miscible extract on cooling to 0° C. gave a small solid deposit.

The data are set out in Table XI and graphed in Diagram 3. They express the mean amount of oil found for each 5 cm. of height from the bottom to the level of the cream. The data in Table XI and the curves of Diagram 3, confirm that the stability rises as the amount of ammoniated Agral W.B. is increased in the water-miscible extract, since the average gradient for the change of oil concentration with height becomes less and less steep as one passes from the 5 per cent. to the 40 per cent. mixture. There was no visible creaming in the 40 per cent. mixture, whereas the 5 per cent. not only creamed but to a large extent separated out clear oil on the surface. In the layers just below the cream

the concentration of oil in all the mixtures was approximately the same and the gradients all become very flat. The 40 per cent. mixture obviously gives an emulsion approximating to complete stability; there has, however, been a slight separation, for although no visible creaming was noted, the gradient is slightly steeper at the very beginning and the end of the curve. The curve for the 5 per cent. mixture is sigmoid in shape, due to the nature of distribution of globule size. The data and gradients of separation indicate that the emulsions prepared from the water-

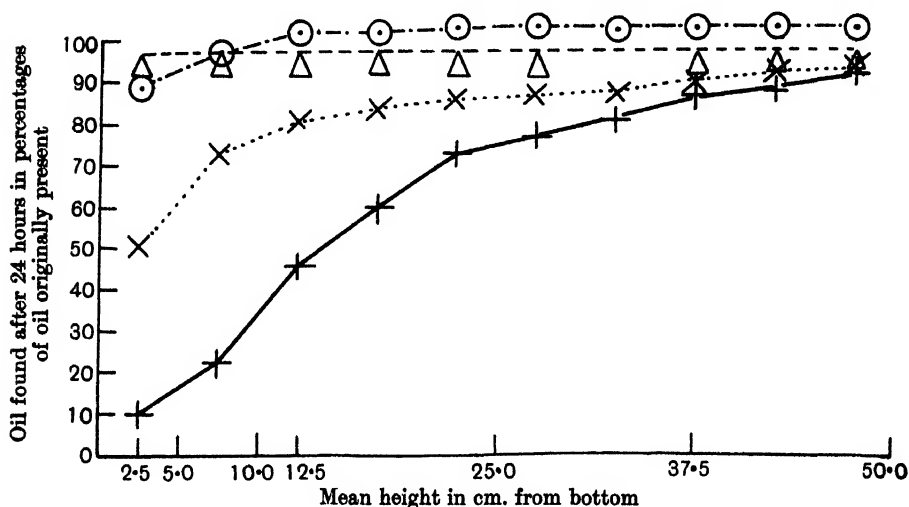


Diagram 3. Permanence of emulsions prepared from Agral W.B. and pyrethrum petroleum spirit extracts.

Mean percentages of oil found in each 5 cm. of height from bottom, after 24 hours.

+	+	Miscible oil contains	5 %	ammoniated Agral W.B.
x	x	"	10 %	"
o	o	"	20 %	"
Δ	Δ	"	40 %	"

In each case 6 c.c. of the miscible oil was shaken for half a minute with water of 30° total calcium hardness which contained in solution 0.5 % Agral I and 0.1 %  $\text{Na}_2\text{CO}_3$ .

miscible extracts containing 10 and 20 per cent. of ammoniated Agral W.B. in the way described should be stable enough for application as sprays on a large scale. This would be particularly true if the tanks were stirred. With respect to the 5 per cent. mixture, no expression of opinion as to its utility is possible until further information is forthcoming as to the relative effectiveness of quick-breaking and stable emulsions.

*The effect of different concentrations of calcium and magnesium salts.* By adding magnesium and calcium sulphates to Harpenden tap-water artificial waters of varying degrees of hardness were obtained. The results with magnesium present are shown in Table X, nos. 21-24. Experiments

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were made with waters containing from 0 to 100° hardness as magnesium and 0.1 per cent.  $\text{Na}_2\text{CO}_3$  and a pyrethrum solution containing 40 per cent. Agral W.B. and 5 per cent. ammonia was used. The results, which are also illustrated in Diagram 4, show that the stability rapidly fell with increasing magnesium concentration. Although the pH may have changed by adding magnesium sulphate, this probably did not influence the results appreciably, as it was found with the highest concentration of magnesium (100° hardness) that doubling and trebling the concentration of sodium carbonate had little effect on the results (Table X, nos. 24 and 26, 27 and 28).

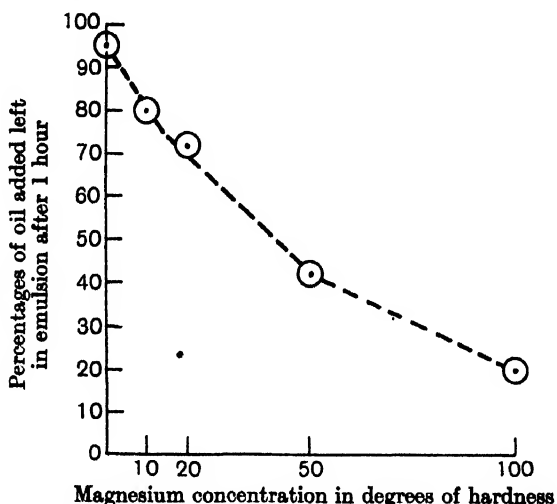


Diagram 4. The effect of magnesium on the separation of emulsions.

Tap-water of 30° total calcium hardness containing 0.1 %  $\text{Na}_2\text{CO}_3$  and increasing amounts of magnesium sulphate.

Increasing the calcium hardness of the tap-water to 100° was without effect on the stability provided the sodium carbonate was increased from 0.1 per cent. to 0.2 per cent.; this can be seen by comparing tests nos. 17, 30 and 31, Table X. With water having 100° hardness as calcium the amount of oil remaining in suspension after 1 hour was 75 per cent. with 0.1 per cent. of added sodium carbonate and 94 per cent. with 0.2 per cent., the value for water containing 30° hardness and 0.1 per cent. sodium being 95 per cent. This result was not unexpected as the added calcium sulphate must have decreased the hydroxyl ion concentration and increased the amount of calcium in solution, conditions which have been shown by Hobson to increase their interfacial tension.

*Miscellaneous results.* By adding 0.1 per cent. sodium hydroxide to water containing 100° hardness as magnesium, an excellent emulsion was at first obtained, but a precipitate of magnesia separated and carried the oil to the surface. As magnesium oxalate is relatively little ionised, the effect of adding ammonium oxalate was tried and found of no value. These results are shown in Table X, nos. 25, 29.

Soft soap in 0.5 per cent. concentration failed to replace the mixture of 0.1 per cent. sodium carbonate and 0.5 per cent. Agral I added to the tap-water (Table X of 32, 15). A mixture of 0.5 per cent. soft soap and 0.1 per cent. sodium carbonate was more effective in emulsifying than 0.5 per cent. Agral I and 0.1 per cent. sodium carbonate (Table X and 33, 15), but it must be remembered that the former mixture renders the solution more alkaline.

*Turkey-red oil preparations.* The samples used for testing the stability of their emulsions in water were those employed for toxicity tests, and their preparation is described on p. 221. The Gerber-tube method is hardly so satisfactory with these preparations owing to the rather great solubility of turkey-red oil in water—but it was thought advisable to carry out strictly comparative tests for both calcium and magnesium-hard waters. Preliminary data for samples A and B (p. 221) are given in Table X, nos. 34–40.

Mixtures A and B (p. 221) prepared from neutral turkey-red oil (with no addition of oleic acid) are noticeable for the ease with which they mix with hard waters of both types and the readiness with which by gentle stirring they can be kept thoroughly incorporated with water. When oleic acid is used to neutralise the alkalinity, characteristic of commercial turkey-red oil, this no longer holds, and the addition of alkali to the aqueous phase is requisite. Figures showing the gradient of separation for mixtures A and C (p. 221) for various types of dispersion media are given in Table XII.

For mixture A (p. 221) (set out under series B in Table XII), the permanence of the emulsion for various types of water is shown by these figures to be quite high and the addition of materials to the dispersion medium for purposes of lowering surface tension is not requisite.

For mixture C (p. 221) (set out under series A in Table XII) the ease of mixing and the permanence of the resulting emulsion is considerably modified by the addition to the water of sodium carbonate or of some material lowering surface tension. In the case of hard water of the type used in these experiments (26° temporary hardness) the addition of 0.1 per cent. sodium carbonate made little difference to the

stability of the emulsion, but a further addition of 0.5 per cent. Agral I to the water phase greatly added to the stability. Greater permanence could also be achieved by increasing the proportion of sodium carbonate to 0.175 per cent., and by a further increase to 0.25 per cent. almost complete stability was established, though this would hardly be required in practice.

Table XII. *Degree of separation of pyrethrum petroleum spirit emulsions containing turkey-red oil.*

Mean amount of oil found after 24 hours in each 5 cm. of height from bottom expressed in terms of the amount found immediately after mixing in *percentages*. 6 c.c. of miscible oil shaken well with 295 c.c. of aqueous medium for 30 seconds.

Series A. Miscible oil contains alkaline turkey-red oil, oleic acid, petroleum spirit (Mixture C, p. 221).

Series B. Miscible oil contains neutral turkey-red oil, petroleum spirit (Mixture A, p. 221).

The tap water used had a temporary hardness of 26°, total hardness 30°.

Dispersion media							
Height in cm.	Tap water	Tap water + 0.1 % Na <sub>2</sub> CO <sub>3</sub>	Tap water + 0.1 % Na <sub>2</sub> CO <sub>3</sub> + 0.5 % Agral I	Tap water + 0.175 % Na <sub>2</sub> CO <sub>3</sub>	Tap water + 0.25 % Na <sub>2</sub> CO <sub>3</sub>	Water of Ca hardness 30°, Mg hardness 30° + 0.25 % Na <sub>2</sub> CO <sub>3</sub>	Water of Ca hardness 30°, Mg hardness 30° + 0.5 % Agral I
		0.1 % Na <sub>2</sub> CO <sub>3</sub>	0.5 % Agral I	0.175 % Na <sub>2</sub> CO <sub>3</sub>	0.25 % Na <sub>2</sub> CO <sub>3</sub>	Na <sub>2</sub> CO <sub>3</sub>	Agral I
<i>Series A:</i>							
0-5	1.6	3.2	52	13.2	82.35	22.1	—
5-10	3.2	3.2	63.7	32.1	94.1	29.1	—
10-15	3.2	4.8	71.2	41.5	101.9	36.3	—
15-20	4.8	—	76.8	50.1	—	40.0	—
20-25	4.8	4.8	82.3	58.5	104.9	43.6	—
25-30	4.8	4.8	85.1	61.3	106.9	44.6	—
30-35	4.8	6.4	87.6	66.1	106.9	46.3	—
35-40	6.4	8.0	91.6	69.7	106.9	48.2	—
40-45	6.4	6.4	92.8	73.6	106.9	51.8	—
45-50	6.4	8.0	93.4	73.6	109.8	51	—
50-	6.4	—	—	—	116.6	This mixture reverted to water-in-oil type	—
Mean of two determinations							
<i>Series B:</i>							
			(0.5 % Agral I No Na <sub>2</sub> CO <sub>3</sub> )			(No Na <sub>2</sub> CO <sub>3</sub> )	
0-5	75	—	100	—	—	89.2	90.2
5-10	80	—	"	—	—	91.9	91.3
10-15	83.7	—	"	—	—	94.6	96.7
15-20	87.5	—	"	—	—	96	96.7
20-25	85	—	"	—	—	96	98.9
25-30	90	—	"	—	—	96	98.9
30-35	90	—	"	—	—	96	100
35-40	90	—	"	—	—	96	101
40-45	88.7	—	"	—	—	96	101
45-50	90	—	"	—	—	96	101
50-	90	—	"	—	—	100	101



The admixture of oleic acid with turkey-red oil enables a larger amount of petroleum spirit to be incorporated with the miscible oil. It has, however, the disadvantage in mixture C (p. 221) of rendering the resulting emulsion quite unsuitable in magnesium-hard waters, the water-in-oil type being formed. This effect is in marked contradistinction to the miscible oils prepared from pure turkey-red oil (mixture A, series B, Table XII), which showed an almost complete stability for 24 hours in water of 30° calcium and 30° magnesium hardness.

The results in Table XII are plotted in Diagram 5.

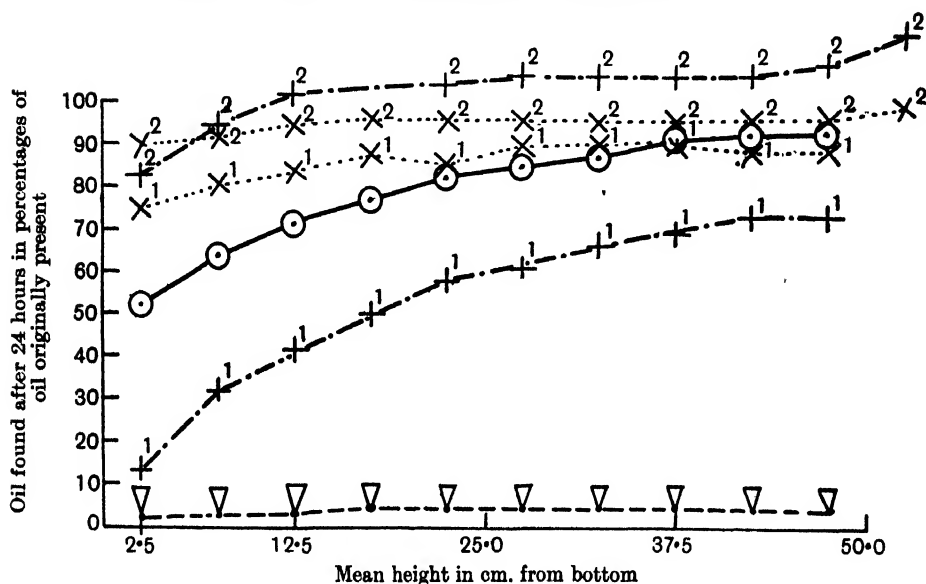


Diagram 5. Permanence of turkey-red oil pyrethrum extract emulsions. Mean percentages of oil in each 5 cm. of height from bottom after 24 hours.

• — — — •	Mixture C (p. 221) in tap water	
▽ — — — ▽	"	+0.1 % $\text{Na}_2\text{CO}_3$
+ <sup>1</sup> — — + <sup>1</sup>	"	+0.175 % $\text{Na}_2\text{CO}_3$
+ <sup>2</sup> — — + <sup>2</sup>	"	+0.25 % $\text{Na}_2\text{CO}_3$
○ — — — ○	"	+0.1 % $\text{Na}_2\text{CO}_3$ + 0.5 % Agral I
x <sup>1</sup> .....x <sup>1</sup>	Mixture A (p. 221) in tap water	
x <sup>2</sup> .....x <sup>2</sup>	"	magnesium-hard water

### DISCUSSION AND CONCLUSIONS.

A water-miscible oil containing an extract of pyrethrum must obey the following conditions:

A. The interfacial tension of the oil, or extract against the aqueous phase must be of a low order, otherwise the resistance to spontaneous emulsification may be too great. This aspect of the problem has been

considered in detail by Hobson (*loc. cit.*). In the case of pyrethrum extracts, the easy dispersion of the oil in the aqueous phase can be obtained by rendering the aqueous phase alkaline, and particularly by the addition to it of materials lowering surface tension, such as soft soap in soft waters, or in calcium-hard waters a reagent such as saponin or the commercial product Agral I, especially if the latter is rendered slightly alkaline. Water-miscible oils in which have been incorporated neutral turkey-red oil (sulphonated castor oil) show this property not only in the case of soft and calcium-hard waters, but also with magnesium waters without the addition to the water phase of materials tending to lower surface tension. A commercial product known as Agral W.B. when incorporated with the miscible oil accentuated the readiness of miscibility, particularly if, wholly or in part, combined with ammonia. Water-miscible oils containing Agral W.B., ammoniated or otherwise, usually require the surface tension of the aqueous phase to be lowered and to be alkaline as indicated above.

B. Readiness of admixture does not necessarily imply that the resulting emulsion is stable. For this purpose a separate stabilising material has to be added, or conditions have to be arranged so that one of the products used in accentuating the miscibility of the oil shall be present in such a form and in such an amount as to secure stability. Such emulsions must have a degree of stability sufficiently great to admit of their ready application with gentle stirring. In the case where Agral W.B. is used our data show that stability is increased by the incorporation with it of a strong ammonia solution in certain proportions. Increase in the amount of ammoniated Agral W.B. to a certain limit tends towards greater stability, a mixture of pyrethrum extract in petroleum spirit to which had been added 10 or 20 gm. per 100 c.c. ammoniated Agral W.B. have a relatively high stability in hard water containing 0.5 per cent. Agral I and 0.1 per cent. sodium carbonate. With magnesium-hard waters there is so great a reversion of phase that many emulsifiers are rendered useless. For such water we have found neutral turkey-red oil mixtures are very suitable. Light petroleum and lubricating oil preparations mix readily with hard water containing alkaline Agral I if 10 per cent. ammoniated Agral W.B. 
$$\frac{0.9 \text{ ammonia in c.c.}}{\text{Agral W.B. in gm.}} = 0.1$$

be incorporated; but some care in balancing the various ingredients is required or the miscible oil may separate out into two phases, particularly on cooling.

C. The proportion of the pyrethrins to solvent must be sufficiently

high to give on dilution a strength requisite to kill the pest and a concentration of oil which shall be low enough to do no damage to foliage. This is a matter of great importance and demands a careful balancing of the mixture. If fixed fatty oils are used in addition to or in the place of petroleum solvents it is probable that greater latitude may be allowed. In the experiments described which only deal with petroleum solvents, the petroleum derivatives rarely if ever exceeded 0.5 per cent. in the strongest diluted spray. A grave damage can be done to foliage by the application of petroleum oils and spirits. Something of the order of 1 per cent. should be fixed as an upper limit for the amount of them allowable in the diluted pyrethrum sprays; actually with smaller quantities than this we have noted an accentuation of the toxicity of pyrethrum, even in cases where the oil used by itself in the same concentration produced little or no lethal effects.

D. The miscible oil mixture must be relatively stable, *i.e.* must not separate out into two liquid phases—or become very turbid on cooling to 0° C. Absolute clarity under these conditions in the case of concentrated pyrethrum preparations is not easy to obtain, and the separation of a small amount of precipitate is not a grave matter, provided it does not lead to the formation of a second liquid phase.

E. The active principles of pyrethrum should be stable in the solution, and they must not suffer such chemical change as to lead to any great loss of toxicity in several months. Our experiments lead us to believe that extracts of pyrethrum made with alcohol (95 per cent.) and petroleum solvents maintain their toxicity over many months in temperate climates or at temperatures of 28° C.; but it is probable that in tropical regions some care in storage would be requisite and that the period should not be too prolonged. In our experiments with extracts containing neutral turkey-red oil or Agral W. B. in concentrations up to 20 per cent. the storage properties were relatively good, and our data, so far, indicate that the combination of a certain proportion of ammonia with the Agral W.B. for purposes of improving the emulsification properties has no deleterious action on toxicity in a period of some months.

It must, however, be noted that the water-miscible petroleum extracts of pyrethrum were not found to be superior to extracts of pyrethrum made with 95 per cent. alcohol either in the degree of permanence of their toxicity or in the stability of their emulsion in water.

We desire to express our thanks to Mr C. T. Gimingham for help and advice during the course of this investigation, and to Mr A. Oggelsby

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and Miss I. Randall for repeated help in carrying out the experiments. Our thanks are also due to the Anglo-Persian Oil Co., Ltd., and to Imperial Chemical Industries for the provision of certain of the materials used in this investigation.

### SUMMARY.

1. Pyrethrum flowers (*Chrysanthemum cinerariaefolium*) both as whole heads and as powder retain their insecticidal properties at ordinary temperatures and at 28° C. for considerable periods if stored in closed vessels. If exposed to the atmosphere in a thin layer as finely ground powder there is risk of loss of toxicity.

2. Alcohol and petroleum extracts of pyrethrum retain their toxicity in temperate climates over many months. Alcohol extracts readily give permanent emulsions when added to water; petroleum extracts require the incorporation of an emulsifier.

3. Water-miscible petroleum extracts of pyrethrum can be prepared by the addition of certain materials, such as ammoniated Agraal W.B. and neutral turkey-red oil.

4. A study has been made of the degree of permanence of the active principles in alcoholic and water-miscible petroleum extracts at ordinary British temperatures and at 28° C. and also in emulsions of these extracts in alkaline spray fluids of varying *pH*. The active principles proved more permanent than has been usually supposed.

5. The readiness with which water-miscible petroleum extracts disperse in the aqueous phase and the stability of the emulsions formed under a variety of conditions have been investigated.

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## THE INFLUENCE OF ENVIRONMENTAL CONDITIONS ON THE DEVELOPMENT OF THE ANGULAR LEAF-SPOT DISEASE OF COTTON. II. THE INFLUENCE OF SOIL TEMPERATURE ON PRIMARY AND SECONDARY INFECTION OF SEEDLINGS

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IN an earlier paper (4) were given the results of a preliminary investigation of some of the environmental factors which influence the development of the disease of cotton variously known as "Blackarm," "Angular Leaf-Spot," "Bacterial Boll-Rot," etc., caused by *Bacterium malvacearum* E.F.S. (*Pseudomonas malvacearum*). It was shown that the conditions of air temperature and humidity each influenced the development of the disease resulting from artificial inoculation of young plants, and that the factors were interrelated in the sense that a change in one allowed of a change in the other without effect on the disease. No attempt was made to differentiate between soil and air temperature, although it was recognised that such a distinction must be made in a more complete investigation of the problem. This was especially true in view of the results of Massey (2) who, working in the Sudan, claimed that the development of the disease in the seedling stage was confined to a definite range of soil temperature, namely from 11° C. to 28–30° C., definite immunity being obtained at 32° C. In a later paper (3) the same author produced further evidence of this influence of soil temperature, and recapitulated the evidence for internal infection of seed first suggested by Archibald (1), but elaborating the theory to include systemic infection of the entire plant under certain conditions without external manifestation of disease.

Preliminary attempts by the writer to confirm these results proved negative, but the question was of such obvious importance that a request from the Sudan Authorities to the Empire Marketing Board for a grant to assist the work was acceded to, and under this grant a series of special chambers was constructed in which soil temperature, air temperature, air humidity and illumination could be independently controlled. A detailed account of the apparatus has been published (5), and it is unnecessary to give full details here. Each unit consists essentially of a

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heat-insulated and thermostatically controlled water-tank, in which the soil tins for the plants are suspended, and fitting over this tank a double-walled glass air-chamber, the temperature and humidity within which are automatically controlled. Illumination is provided by two 500-watt lamps in suitable reflectors suspended over each case, the heat from the lamps being absorbed by a continuously flowing water film. Cotton seedlings have been found to grow satisfactorily in these chambers, although owing to the entirely overhead character of the lighting and its low intensity compared with tropical sunlight they become somewhat "drawn."

The influence of several factors has been investigated to some extent, but only those experiments dealing with the effect of soil temperature on seedling infection will be described here. Later papers will deal with the other factors, especially with regard to their influence on secondary infection by spray inoculation.

It should be pointed out that so far no attempt has been made to deal with the extremely difficult problem of automatic control of soil moisture. Throughout the experiments the plants were supplied with water of the right temperature from time to time as thought necessary. Examination of the soil at the end of the experiments showed that in no case had watering been so excessive as to cause any degree of waterlogging. While it is recognised that soil moisture may have some influence on the disease either directly or indirectly through its effect on the plant, the consistency of the results with different types of soil and the fact that the moisture was kept well within the extremes of dryness and wetness makes it improbable that the results will be seriously affected by variations in this factor.

The seed used throughout the experiments was "Sakellarides" variety from the Gezira Plain, supplied by the courtesy of Mr R. E. Massey, Botanist to the Sudan Government. Different lots of seed were used in each experiment, but all the seed was derived from heavily infected plants, and hence presumably carried the organism.

### DESCRIPTION OF EXPERIMENTS.

#### *Influence of soil temperature on primary infection.*

*Exp. 1.* Four different treatments were used in the experiment, and as there were eight tins in each chamber, each treatment was duplicated at every temperature. Ten seeds were sown per tin, giving a possible twenty seedlings per treatment at each temperature. The treatments were as follows:



(a) Seed delinted in concentrated sulphuric acid for 15 minutes, washed, immersed in 1 : 500 mercuric chloride for 15 minutes, and again washed.

(b) Seed soaked in a very heavy suspension in distilled water, from a pure culture of a virulent strain of *B. malvacearum*.

(c) A small portion of the testa of each seed carefully chipped off from the side without injury to the embryo and the seed then soaked in a suspension of the organism.

(d) Seed soaked in sterile water, but otherwise untreated.

In each case the vessel containing the seed and disinfectant, suspension, or water respectively was evacuated for a short time to ensure complete wetting of the seed. After treatment the seed was sown directly in the tins at a depth of about 4 cm., at the following range of temperatures: 15° C., 19° C., 23° C., 27° C., 31° C., 35° C. The soil used was a rich glasshouse compost containing one-fourth of its bulk of sand. The temperature in all the air-chambers was maintained at 25° C. and the humidity at an average of 75 per cent. As soon as the seedlings appeared above the soil the artificial lighting was provided, the time-switch being set to give 16 hours' illumination daily.

Germination was good at all temperatures except 15° C., where the seed germinated irregularly and sparsely. The times required for germination at the different temperatures are shown in Table I, where the time given refers to the first appearance of seedlings above the soil.

Table I.

*Time (in days) for germination and germination percentage.*

	15° C.	19° C.	23° C.	27° C.	31° C.	35° C.
Time for germination	5-6	5	3	2½	2	3
Total no. germinated	25	54	73	66	67	74
Germination %	31.3	67.5	91.3	82.5	83.8	92.5

The "number germinated" and "germination percentage" given in Table I include all seed irrespective of treatment, that is, a total of eighty seeds at each temperature.

Infection was apparent a few days after germination, and an examination of the seedlings was made 14 days after sowing except in the cases of chambers numbers I and VI (19° C. and 15° C.), when the examination was made 19 days from the date of sowing. Infection of the cotyledon takes the form of water-soaked spots which are usually most numerous around the edges of the cotyledon. Any seedling showing one

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or more definite lesions of this type was counted as infected, and in Table II the numbers of such infected seedlings are given for the duplicate tins of each treatment, together with the percentage infection for the sum of these numbers.

Table II.

*Exp. 1. Percentage of infection at the different temperatures for the four treatments.*

	Soil temperature						Average % for 5 temps.
	15° C.	19° C.	23° C.	27° C.	31° C.	35° C.	
Sterilised externally.							
I. No. of seedlings	6	6	10	9	9	10	}
No. infected	0	0	0	0	0	0	
II. No. of seedlings	8	8	10	10	10	10	
No. infected	0	0	0	0	0	0	
Average %	0	0	0	0	0	0	
Untreated.							
I. No. of seedlings	2	9	10	8	8	10	}
No. infected	0	2	1	3	1	0	
II. No. of seedlings	5	6	9	9	1	9	
No. infected	0	1	0	1	0	0	
Average %	0	20	5.2	23.5	6.7	0	
Inoculated plain.							
I. No. of seedlings	1	8	10	9	9	10	}
No. infected	?	2	0	3	2	2	
II. No. of seedlings	2	8	10	10	10	10	
No. infected	?	2	2	2	3	2	
Average %	?	25	10	26.3	26.3	20	
Inoculated chipped.							
I. No. of seedlings	0	6	8	6	7	7	}
No. infected	?	4	3	6	6	4	
II. No. of seedlings	1	3	8	5	7	8	
No. infected	?	3	6	5	4	6	
Average %	?	78	56	100	71.5	66.6	
Average % for three treatments where in- fection occurred	?	35	24	42.6	33.4	26	

As mentioned previously, the seeds at a soil temperature of 15° C. germinated very irregularly and made exceedingly poor growth. The only exception was in the case of the externally sterilised seed, of which six and eight respectively out of ten germinated. No infection was apparent on the seedlings which did appear, but in view of the fact that the sterilised seed germinated more or less normally, there is a strong presumption that the poor germination of the inoculated seed was due to infection. This cannot, however, be stated with certainty. In a later

experiment (see Exp. 2) severe infection was obtained at 15° C., and this lends support to the hypothesis. In view of this uncertainty as to the cause of the poor germination at this temperature, therefore, this column of figures has been omitted in calculating the average percentage infections for each treatment.

It will be seen from Table II that the most striking result is that due to variation in seed treatment. No infection in any case was found on the seedlings produced from seed which had been externally sterilised. Infection was low in the case of the untreated seed, but sufficiently high to show that, under the given set of air conditions, an appreciable amount of disease may develop. The presumption from these two results would appear to be that, for this batch of seed at least, disease arose only from organisms carried on the outside of the seed. Where the organism was present in much larger numbers ("inoculated plain") a greater amount of disease was produced, whilst where the organism was introduced actually within the seed-coat and hence in contact with the embryo from the beginning of germination, very severe infection resulted. In this connection a factor which cannot be shown numerically should be taken into account, namely, the severity of attack. In the case of the inoculated chipped seed the infection was very severe, often involving the entire cotyledon, and in some cases resulting in the death of the seedling. With the plain inoculated seed the attack of individual plants was less severe, but still very conspicuous. With the untreated seed, on the other hand, the infection was usually very much less in degree, often being limited to one or two spots on the edge of the cotyledon. The variation in degree of infection was thus more marked than the figures in Table II show.

With regard to the effect of soil temperature, it will be seen that, while there appears to be some influence, this is less marked than the difference between treatments. A marked falling off in degree of infection is apparent at the higher temperatures, and this is true for each treatment. Nevertheless, it is clear that even so high a soil temperature as 35° C. is insufficient to inhibit the development of the disease except on the untreated seed.

*Exp. 2.* The plan of this experiment was similar to that of Exp. 1. The same four treatments were applied, but in this case, in order to ascertain whether a still higher temperature would inhibit the disease, a wider range of soil temperatures was used, namely, 15° C., 20° C., 25° C., 30° C., 35° C., 40° C. The only other differences in the two experiments were that, in this case, in place of glasshouse compost, cotton-growing soil obtained from the Gezira Plain in the Sudan was used, and

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that the air conditions were set at 27° C. and an average of 85 per cent. humidity.

The seed was sown slightly more deeply than in the previous case, and this accounts for the longer period before the appearance of the seedlings. Germination was good and rather more even than in the previous experiments. The numbers germinating out of eighty seeds sown are given in Table III.

Table III.

*Percentage germination at different temperatures (Gezira soil).*

	15° C.	20° C.	25° C.	30° C.	35° C.	40° C.
Time for germination (days)	14	8	5	5-6	5	7
Total no. germinated	37	53	60	55	42	29
Germination %	45.3	66.3	75.0	68.8	52.5	36.3

Table IV.

*Exp. 2. Percentage of infection at the different temperatures for the four treatments.*

	Soil temperature						Average % for 6 temps.	
	15° C.	20° C.	25° C.	30° C.	35° C.	40° C.		
Sterilised externally.								
I. No. of seedlings	6	8	10	10	8	2	}	0
No. infected	0	0	0	0	0	0		
II. No. of seedlings	10	9	10	10	9	7		
No. infected	0	0	0	0	0	0		
Average %	0	0	0	0	0	0		
Untreated.								
I. No. of seedlings	1	7	8	8	5	4	}	39.7
No. infected	0	5	6	3	1	0		
II. No. of seedlings	3	4	8	7	6	7		
No. infected	2	0	5	3	2	0		
Average %	50.0	45.4	68.8	40.0	27.2	0		
Inoculated plain.								
I. No. of seedlings	5	5	9	5	6	2	}	63.6
No. infected	4	2	6	5	3	0		
II. No. of seedlings	5	8	7	8	3	3		
No. infected	4	8	4	3	2	1		
Average %	80.6	77.0	62.5	61.5	55.6	20.0		
Inoculated chipped.								
I. No. of seedlings	3	4	6	2	1	2	}	92.4
No. infected	3	4	6	2	1	2		
II. No. of seedlings	4	8	2	4	2	1		
No. infected	4	8	2	2	2	1		
Average %	100.8	100	100	67.7	100	100		
Average % for three treatments where in- fection occurred	81.0	75	72.5	53.0	47.8	21		

Infection was more severe than in the earlier experiment, and 2 weeks after sowing was apparently completely developed. Examination of the seedlings was made 17 days after sowing, except in the case of the seedlings at a soil temperature of 15° C. when a further 7 days was allowed for full development of infection owing to the delayed germination. The results are given in Table IV.

It will be seen that the results of this experiment fully bear out those of the previous trial. Again no infection was found on the externally sterilised seed at any temperature, while some infection occurred in all other cases except on the untreated seed at 40° C. The effect of temperature in this experiment is more marked, there being a steady fall in average infection with increasing temperature when the three treatments in which infection resulted are considered together. A brief discussion of the results of these two experiments is given later in this paper.

*The influence of soil temperature on secondary infection.*

In order to test whether soil temperature would have any influence on the susceptibility of the plant to external (secondary) infection and, at the same time, to endeavour to obtain confirmatory evidence for the theory of possible latency and spread of the organism within the plant, a further experiment was carried out on the plants in Exp. 1. The plants were allowed to grow on for a week after the examination for primary infection had been made, by which time they had reached a height of about 18 inches and had produced several true leaves. These newly formed leaves were entirely free from disease. Four tins, one of each seed treatment, were then removed from each tank and the plants thoroughly sprayed, by means of an atomiser, with a strong suspension of *B. malvacearum* in water. They were returned to the chambers, and allowed to remain under the same conditions as before, except that the relative humidity was increased to 85-90 per cent. to provide the best possible conditions for infection. The sprayed plants occupied the left half of each chamber, the four tins of plants in the right half being left unsprayed. After 18 days all the plants were examined for infection individually<sup>1</sup>. The amount of infection was very variable between different plants of the same tin, and an estimate of the degree of infection was difficult to obtain. Finally the procedure was adopted of grading the plants in three classes which are shown in columns 5, 6, and 7 of Table V. A plant showing a few scattered spots only was classed as "lightly infected"; up to fifteen lesions on a plant constituted "moderate

<sup>1</sup> This examination was made by Dr W. B. Brierley.

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infection," while a greater amount of disease was estimated as "heavy infection."

Table V.

*Influence of soil temperature on secondary infection.*

Soil temp.	Treatment	No. of plants	Infection			
			None	Light	Moderate	Heavy
15° C.	Sterilised <sup>1</sup>	7	—	3	1	0
	Untreated	0	—	—	—	—
	Inoculated:					
	Plain	1	—	1	—	—
19° C.	Chipped	0	—	—	—	—
	Sterilised	5	0	4 <sup>2</sup>	1 <sup>3</sup>	0
	Untreated	8	2	2 <sup>4</sup>	3	1
	Inoculated:					
23° C.	Plain	8	2	2	3	1
	Chipped	4	1	2	1 <sup>5</sup>	0
	Sterilised <sup>6</sup>	9	2	4	1	0
	Untreated	10	1	6 <sup>7</sup>	1	2
27° C.	Inoculated:					
	Plain	10	4	5	0	1
	Chipped	7	1	5	1	0
	Sterilised <sup>8</sup>	9	0	4	1	2
31° C.	Untreated	8	4	1	0	3
	Inoculated:					
	Plain	10	0	6	2	2
	Chipped	4	0	1	2	1
	Sterilised	4	0	3	0	1
	Untreated	5	0	2	2	1
	Inoculated:					
	Plain	7	0	3	3	1
	Chipped	6	0	4	1	1

<sup>1</sup> Plants very small with much reduced leaves.

<sup>2</sup> One plant with numerous doubtful lesions.

<sup>3</sup> Plus numerous doubtful lesions.

<sup>4</sup> One plant with numerous doubtful lesions.

<sup>5</sup> Plus numerous doubtful lesions.

<sup>6</sup> Two remaining plants with doubtful lesions.

<sup>7</sup> One plant with numerous doubtful lesions.

<sup>8</sup> Two remaining plants with doubtful heavy infection on one leaf of each.

On the basis outlined the infection at 15° C. appears to be very slight, but it should be borne in mind that there were only eight sprayed plants altogether, and that these were extremely small with only one or two very reduced leaves. Thus the infection in this case was relatively as severe as in any of the other chambers.

It will be seen from Table V that it is impossible to detect any marked effect in degree of infection due either to soil temperature or to previous seed treatment. There appears to be somewhat more infection at 27° C. soil temperature, but this is hardly significant. Infection was irregular but widespread and occurred at all soil temperatures.

The unsprayed plants showed no infection except in one or two cases where a leaf touched one of the sprayed plants, when infection was transmitted by contact to this leaf. The significance of this lack of infection will be discussed later.

#### DISCUSSION OF RESULTS AND LABORATORY EXPERIMENTS.

Several points are apparent from a study of the results of the experiments described. With regard to the location of the parasite in the seed, the experiments on primary infection show that, although the seed was derived from diseased plants, thorough external disinfection of the seed results in the production of healthy seedlings showing no sign of the disease. This would appear to indicate that, for these batches of seed at least, the organism was not present in a virulent state within the seed. It might, however, be argued that the treatment with sulphuric acid had stimulated germination (a fact known to be the case) and thus enabled the seedlings to resist infection. That this is the explanation is not borne out, however, by laboratory experiments on this same seed. Samples were taken and subjected to a more vigorous sterilisation than can be achieved by sulphuric acid and mercuric chloride alone. All cotton-seed bears at the micropylar end a very close tuft of short fuzz which is difficult to wet, and is not completely removed even by 15 minutes' treatment with sulphuric acid. In the tests to be described this tuft was removed by scraping each seed individually with a scalpel under a dissecting microscope until the exterior presented a clean surface free from any hairs. The seed was then sterilised in 1 : 500 mercuric chloride under the vacuum pump, washed in sterile water and crushed in sterile broth. Parallel samples were first dehusked, the embryo extracted and lightly sterilised externally and similarly crushed in broth. Platings from these samples showed no colonies of *B. malvacearum* in either case, the dehusked seed producing no organisms whatever, while the former samples gave colonies of a number of different saprophytic bacteria and fungi. Control samples similarly sterilised, but not crushed, produced no colonies. The organisms isolated in the case of the crushed seed had, therefore, apparently originated from a position between the seed-coat and the embryo, or possibly within the micropylar passage.

Taking these results into consideration, it is reasonable to conclude that the seed used in this experiment did not contain the organism within it, although the seed was derived from heavily infected plants in the Sudan. Some infection occurred, however, on the seedlings from untreated seed, and hence the conclusion is reached that the organism was

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present on the exterior of the seed or in the fuzz. The heavy infection resulting from inoculation of the outside of the seed shows that presence of the parasite in this position is sufficient to account for severe damage. It would appear that more extensive trials in the field with various methods of external disinfection of seed might be well worth while.

So far as the effect of soil temperature is concerned, the experiments described do not entirely accord with the results given by Massey. Infection can occur at all soil temperatures at which growth of the plant is possible, the air temperature being 25°–27° C. A falling-off in amount is noticeable at temperatures above 30° C., but this is not sufficient in degree to render it likely that sowing of seed at the time of high soil temperature will result in effective control. Low temperatures, on the other hand, appear to increase the amount of infection, even when carried below a temperature at which the plant can make reasonable growth.

Considering now the experiments on secondary infection, it is seen that neither soil temperature nor seed treatment has had any marked influence on infection resulting from spray inoculation. This result is hardly surprising, since the attack of a local parasite on the purely aerial parts of the plant could only be indirectly affected by soil temperature through the effect of this factor on the metabolism and growth of the host. If, however, the theory of possible latency and internal spread of the organism were correct, it would be expected that the unsprayed plants which had been infected in the cotyledonary stage would show further infection when placed under these conditions demonstrably suitable for development of infection. That this was not so indicates that under these conditions at least internal spread had not taken place.

The accident that a few leaves of the unsprayed plants touched some of the leaves of the sprayed and became infected at these points confirms the view that infection is easily transmitted by contact under humid conditions.

### SUMMARY.

Experiments on the angular leaf-spot disease of cotton carried out under controlled conditions lead to the following conclusions:

1. Seed derived from diseased plants may give rise to infected seedlings.
2. This infection is due to bacteria carried on the outside of the seed and in the fuzz.
3. Thorough disinfection of the exterior of the seed results in healthy seedlings.



4. The amount of primary infection resulting from infected seed decreases at soil temperatures above 30° C., but infection is not inhibited at 40° C.

5. Soil temperature has little or no effect on secondary infection resulting from spray inoculation of the plants.

6. Plants diseased in the seedling stage grow out free from disease, if no further inoculation occurs.

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# THE INFLUENCE OF ENVIRONMENTAL CONDITIONS ON THE DEVELOPMENT OF THE ANGULAR LEAF-SPOT DISEASE OF COTTON

## III. THE INFLUENCE OF AIR TEMPERATURE ON INFECTION

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*(With Plate XXXIX and 3 Text-figures.)*

IN the first paper of this series(3) experiments of a preliminary nature were described dealing with the effect of air temperature and humidity on the development of the serious cotton disease variously known as "Angular Leaf-Spot," "Black-Arm" or "Bacteriosis." These experiments, though not extensive, and carried out under comparatively roughly controlled conditions, indicated the importance of these factors in influencing the development of the disease resulting from spray inoculation of young plants. In a later paper(5) an outline was given of the first experiments carried out in the more accurately controlled apparatus constructed under a grant from the Empire Marketing Board, details of which have been published separately(4). These first experiments dealt with the influence of soil temperature, and it was pointed out that while this factor had some effect on primary infection of seedlings, *i.e.* infection resulting from seed inoculation, this effect was not very great within the range of soil temperature at which cotton is usually grown. It was further shown that soil temperature had no detectable effect on secondary infection, *i.e.* infection arising from subsequent external inoculation, as by spraying the plants with a suspension of the organism.

The experiments have now been extended to include the effect of air temperature on such secondary infection of young plants and the results are described in the present paper.

The Rothamsted control chambers(4) consist essentially of heat-insulated and thermostatically controlled water-tanks, in which the soil tins for the plants are suspended and, fitting over these tanks, double-

walled glass air chambers, within which the temperature and humidity are automatically controlled. Illumination is provided by two 500-watt lamps in suitable reflectors suspended over each case, the heat from the lamps being absorbed by a screen of continuously flowing water.

The seed used throughout the experiments has been "Sakellarides" variety from the Gezira Plain, supplied by the courtesy of Mr R. E. Massey, Botanist to the Sudan Government.

#### DESCRIPTION OF EXPERIMENTS.

*Exp. 1.* The forty-eight tins for the six chambers were filled with Gezira cotton soil and sown, in the glasshouse, with Sakel seed, two seeds in each tin. Before sowing the seed was treated with concentrated sulphuric acid for 15 minutes, to sterilise the outside. The plants were allowed to grow for 6 weeks, by which time they had produced two true leaves with two or three unfolding.

The six control chambers were started several days prior to the beginning of the experiment, the thermostats of the soil temperature tanks being set for a temperature of 22° C. (The temperature variation in the tanks is usually about 1° C.) The humidity controls were all set for 85 per cent. relative humidity. This experiment was carried out with an early type of humidity control(2) and the variation in humidity was somewhat great. Throughout the experiment, however, the humidity in all chambers was over 80 per cent. The thermostats of the air chambers were set for the following range of temperatures: 16, 20, 24, 28, 32 and 36° C. Unfortunately, a warm spell of weather began just at the time the experiment was started, and the lower temperatures could not be maintained, especially when the artificial lights were on. In these chambers, therefore, a variation of 4–5° C. resulted, and the average temperature of the six chambers during the experiment was 18–19, 22, 24, 28, 32 and 36° C.

The young plants were thoroughly sprayed by means of an atomiser with a strong suspension in sterile water of a virulent culture of *Bacterium malvacearum*, and placed in the chambers. Two tins in each chamber were left unsprayed as controls. Illumination was given for the rest of that day and night, and thereafter the plants received 16 hours' lighting daily from 5 p.m. to 9 a.m. The illumination was given during the night in order to allow the heating of the room due to the lights to be balanced by the drop in external temperature.

Infection was visible at the higher temperatures in 6–7 days and appeared complete in 12 days. The plants at the three high temperatures

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were accordingly examined after this period and the amount of infection estimated. Infection was slower at the lower temperatures and these plants were left for a longer period, those at 24° C. for 19 days, and the remaining two chambers for 24 days, before final examination.

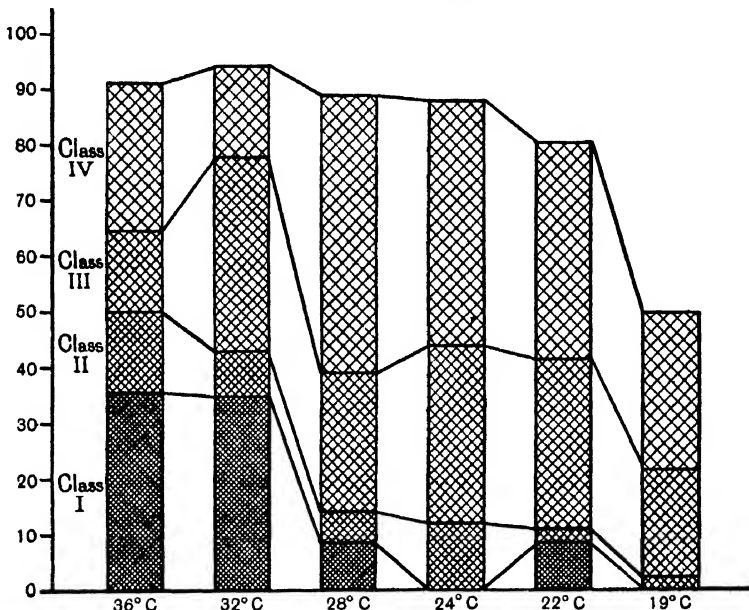
Table I.

*Exp. 1. Distribution of infection at various air temperatures.*

	36° C.				32° C.				28° C.				24° C.				22° C.				19° C.				
	Leaf no.				Leaf no.				Leaf no.				Leaf no.				Leaf no.				Leaf no.				
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	
Plant no. 1	∞	∞	5	5	∞	10	15	1	—	10	7	5	—	—	35	11	—	0	52	10	0	0	15	0	
„ 2	∞	∞	5	1	—	51	30	4	—	0	18	0	—	15	9	1	—	1	11	14	—	2	15	6	
„ 3	∞	5	20	5	∞	∞	20	15	—	4	3	30	—	1	16	20	—	0	42	5	0	4	11	12	
„ 4	∞	60	11	5	∞	—	10	15	—	12	7	5	—	—	16	1	—	—	65	14	0	0	14	0	
„ 5	∞	∞	∞	20	—	—	5	5	—	—	4	4	—	0	5	0	—	7	15	5	0	1	0	0	
„ 6	∞	25	25	4	∞	∞	∞	5	—	3	25	7	—	3	16	15	—	0	65	13	0	4	7	2	
„ 7	∞	47	55	5	∞	75	30	2	—	∞	8	7	—	0	30	1	—	2	8	20	—	0	6	0	
„ 8	∞	36	12	5	—	25	22	0	—	3	18	0	—	1	13	25	—	2	12	15	0	0	20	10	
„ 9	∞	26	25	—	—	12	10	—	—	—	3	4	2	—	3	2	6	—	0	6	9	0	0	13	15
„ 10	15	6	5	0	∞	11	20	10	∞	18	13	5	—	—	—	—	—	0	3	24	8	0	1	7	10
„ 11	∞	∞	18	7	∞	∞	15	0	—	15	20	5	—	—	—	—	—	—	0	5	3	0	0	5	6
„ 12	23	28	0	0	—	—	—	—	—	∞	10	—	—	—	—	—	—	—	0	21	7	0	0	40	5
Total no. of leaves	48				37				36				25				36				46				
Class I																									
50 spots and over	17	(35.4 %)			13	(35.1 %)			3	(8.3 %)			0	(0.0 %)			3	(8.3 %)			0	(0.0 %)			
Class II																									
25 spots and over	7	(14.6 %)			3	(8.1 %)			2	(5.6 %)			3	(12.0 %)			1	(2.8 %)			1	(2.2 %)			
Class III																									
10 spots and over	7	(14.6 %)			13	(35.1 %)			9	(24.8 %)			8	(32.0 %)			11	(30.5 %)			9	(19.6 %)			
Class IV																									
Less than 10 spots	13	(27.1 %)			6	(16.2 %)			18	(50.0 %)			11	(44.0 %)			14	(38.9 %)			13	(28.2 %)			
Total no. of leaves infected	44	(91.7 %)			35	(94.5 %)			32	(88.7 %)			22	(88.0 %)			29	(80.5 %)			23	(50.0 %)			

In the estimation of the disease an examination was made of each leaf, starting from the base of the plant, and counting the number of spots occurring on each separate leaf. A difficulty arises in such a method of estimation in that in many cases neighbouring spots tend to coalesce, forming a patch, in which it is difficult or impossible to distinguish the individual centres of infection (Plate XXXIX, fig. 2). This difficulty becomes even greater in the type of infection characterised by an attack of the leaf tissue on either side of a vein and extending along the vein (Plate XXXIX, fig. 3) (see also (1), Figs. 239 and 241). Clearly, in any attempt to estimate the *severity* of infection, as distinct from the *incidence* of infection obtained by mere counting of the number of diseased leaves, more weight must be given to such an extended lesion than to a single circumscribed spot. An arbitrary method of estimation was finally adopted of counting each patch, either of the "coalescence" type where individual centres of

infection could not be distinguished, or the "extended" type along a vein, as equivalent to five spots. This is obviously not exact, but provided the same method is used throughout, the estimation of severity of attack will be reasonably accurate. The results of this experiment are given in full in Table I. The control plants are not included in the table, as these were free from infection. Where more than about seventy-five spots or the equivalent in patches occurred on a single leaf the infection was recorded as indefinite ( $\infty$ ). In order to obtain an estimate of the total infection and its severity at each temperature the leaves were grouped



Text-fig. 1. *Exp. 1.* Percentage infection in four classes at various air temperatures.

in arbitrarily limited classes: Class I, severe infection, fifty spots or more; Class II, moderate infection, twenty-five spots or more; Class III, light infection, ten spots or more; Class IV, very light infection, less than ten spots. Text-fig. 1 shows the percentage infection in the four classes at the six temperatures. It is apparent that at the two higher temperatures, 32 and 36° C., there is little or no significant difference in infection, the slight reduction in the amount of heavy infection (Classes I and II) at 32° C. being balanced by the increased amount of light infection (Class III). Below 30° C., however, there is a decided fall in the amount and severity of infection with decreasing temperature. From the figures in Table I it is clear that the apparent increase in severe

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infection at 22° C. is hardly significant, the three leaves having over fifty spots only just falling within Class I.

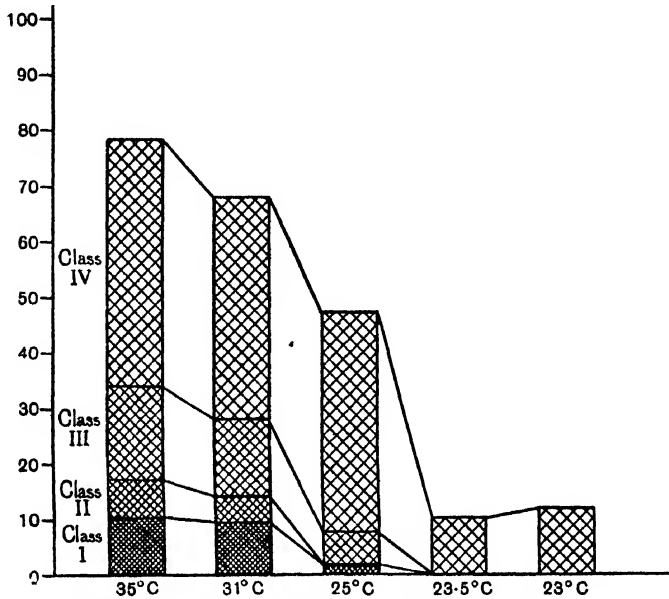
It should be noted here that the plants used were small and at the time of spraying only two true leaves were fully unfolded and even these were still developing. This is of importance in view of the distribution of the disease on the plant. It will be noted from Table I that at the two higher temperatures infection was most severe on the basal leaves, decreasing in intensity on passing up the plant. In later experiments somewhat older plants were used, and in most cases the two or three basal leaves were more or less fully developed. It has been shown by other workers and confirmed by the writer that *B. malvacearum* normally attacks only developing tissues, and this would explain the lack of infection of the basal leaves in later experiments. In the present experiment also it is highly probable that the lack of infection of the basal leaves at the lower temperatures may be due to the slowness of development of the disease at these low temperatures and the consequent maturing of these leaves before the disease has progressed far enough to produce a lesion. Further development is then arrested. Reference to this variation in distribution of the disease will be made in the discussion (p. 532).

*Exp. 2.* This experiment was in essentials a repetition of the first with certain modifications. The plants were raised as before in the glasshouse in Gezira soil with four plants per tin. When three to four true leaves were unfolded the plants were sprayed with a water-suspension of the bacteria and placed in the chambers. The soil-temperature thermostats in all chambers were set for 28° C. and the humidity controls for 85 per cent. relative humidity. The air thermostats were set for the following range of temperatures 40, 35, 30, 25 and 20° C., while one chamber was left unheated to run at air temperature. As in the previous experiment it proved impossible to maintain the lower temperature, while the highest temperature showed a fluctuation from 38 to 40° C. The actual mean temperatures derived from the thermograph charts were therefore, 39, 35, 31, 25, 23.5 and 23° C. Illumination was provided for 16 hours out of the 24.

After 16 days it was found that only a negligible amount of infection had occurred, and it seemed certain that the culture of *B. malvacearum* used had lost a considerable degree of virulence. As the plants had made good new growth during the period in the chambers and most of the basal leaves had dropped off, the plants were re-sprayed with a strong suspension of a newly isolated culture of the same strain which had been maintained on experimental plants in the glasshouse and had thus



retained its full virulence. Good infection resulted, and appeared complete after 14 days at 35, 30 and 25° C. The plants at 39° C. had made little or no growth in the chambers and after this further period of 14 days were dead. The plants at the two lower temperatures were left for a further 7 days to allow of full development of any infection.



Text-fig. 2. *Exp. 2. Percentage infection in four classes at various air temperatures.*

Table II.

*Exp. 2. Distribution of infection in four classes at various air temperatures.*

	35° C.		31° C.		25° C.		23.5° C.		23° C.	
	No. of leaves	%	No. of leaves	%	No. of leaves	%	No. of leaves	%	No. of leaves	%
Total no. of leaves	88	—	75	—	68	—	60	—	51	—
Class I, 50 spots and over	9	10.2	7	9.3	1	1.5	0	0.0	0	0.0
" II, 25 " "	6	6.8	4	5.3	0	0.0	0	0.0	0	0.0
" III, 10 " "	15	17.0	10	13.3	4	5.9	0	0.0	0	0.0
" IV, less than 10 spots	39	44.3	30	40.0	27	39.7	6	10.0	6	11.7
Total no. of leaves infected	69	78.3	51	67.9	32	47.1	6	10.0	6	11.7

Estimation of infection was carried out on the same basis as in the previous experiment. The distribution of the infection into the four arbitrary classes at the various air temperatures employed is summarised in Table II and shown in graphic form in Text-fig. 2. It will

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be seen that, in general, the results of this experiment are the same as in the previous test, infection occurring most freely at temperatures above 30° C. and falling off rapidly in intensity below this point.

*Exp. 3.* In further confirmation of these experiments it may be well to give here the relevant figures of an experiment which will be included at greater length in a later paper. This experiment is one of a series to be carried out dealing with the effect of alternating conditions on the disease. One of the chambers was arranged to give an alternating air temperature, while the remaining five were run at constant temperatures covering the extreme range of this chamber. As the conditions in these five cases were identical with the experiments described above they are relevant to this discussion.

As before the tins were filled with Gezira soil and sown in the glasshouse with four seeds per tin. When of a suitable size the plants were transferred to the chambers for a few days and then sprayed with the organism. The mean temperatures of the five chambers were 39, 35, 30, 25 and 23·5° C. As in *Exp. 2* the plants at 39° C. made no growth and many were dying at the close of the experiment. No definite infection was detectable on the leaves of these plants, and they are omitted in the further discussion.

One further modification in this experiment aimed at testing the influence of time of spraying the plants in relation to the time of illumination. The plants in one half of each chamber were sprayed 30–60 minutes after the lights had been switched on, and the remaining plants on the following morning 30–60 minutes after the lamps were extinguished. As in the previous experiments infection developed rapidly at the higher temperatures and more slowly at the lower. The plants at 35° C. were examined after 21 days and the remainder 3 days later. The results are again summarised in Table III, and shown graphically in Text-fig. 3.

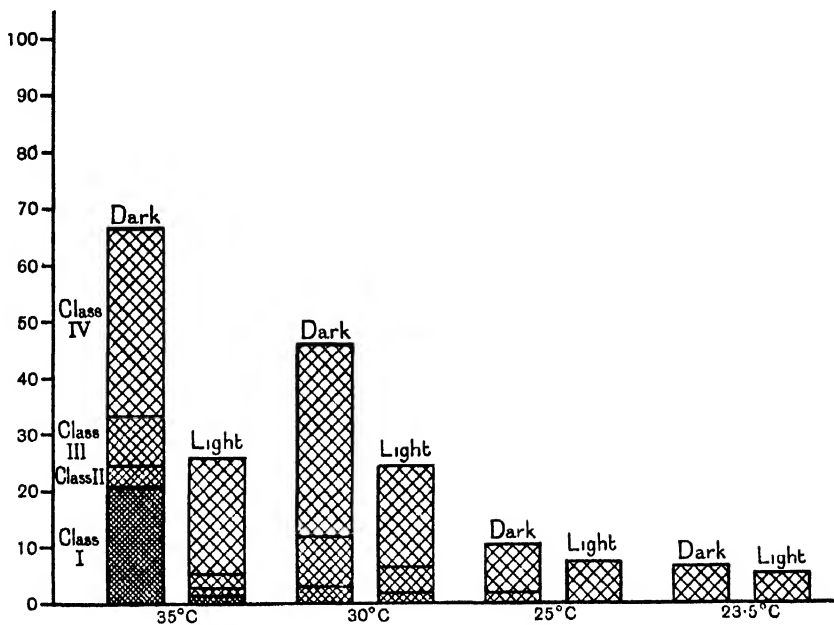
Table III.

*Exp. 3. Distribution of infection in four classes at various air temperatures.*

	35° C.				30° C.			
	Dark		Light		Dark		Light	
	No. of leaves	%	No. of leaves	%	No. of leaves	%	No. of leaves	%
Total no. of leaves	78	—	78	—	68	—	62	—
Class I, 50 spots and over	16	20·5	1	11·3	0	0	0	0
" II, 25 " "	3	3·9	1	1·3	2	2·9	1	1·6
" III, 10 " "	7	8·7	2	2·6	6	8·8	3	4·8
" IV, less than 10 spots	26	33·4	16	20·5	23	33·9	11	17·8
Total no. of leaves infected	52	66·5	20	25·7	31	45·6	15	24·2

Table III (cont.).

	25° C.				23.5° C.			
	Dark		Light		Dark		Light	
	No. of leaves	%	No. of leaves	%	No. of leaves	%	No. of leaves	%
	59	—	55	—	64	—	60	—
Total no. of leaves	0	0	0	0	0	0	0	0
Class I, 50 spots and over	0	0	0	0	0	0	0	0
„ II, 25 „ „	1	1.7	0	0	0	0	0	0
„ III, 10 „ „	5	8.5	4	7.3	4	6.3	3	5.0
„ IV, less than 10 spots	6	10.2	4	7.3	4	6.3	3	5.0
Total no. of leaves infected								

Text-fig. 3. *Exp. 3.* Percentage infection in four classes at various air temperatures.

Considering first the effect of temperature it is clear that the results of this experiment agree very closely with those of *Exp. 2*. There is the same high degree of infection at 35° C. with rapid decrease in the amount of disease at progressively lower temperatures. This is true of both series, considered separately, whether sprayed in the light or in the dark. The comparison between the two series, however, is striking. At all four temperatures a higher degree of infection resulted from spray inoculation in the dark than on the corresponding plants which were sprayed in the light. At the two higher temperatures this influence of time of inoculation is very marked. This point will be discussed later.

## DISCUSSION OF RESULTS.

Comparison of the results of the air-temperature experiments outlined here with those on the influence of soil temperature previously described<sup>(5)</sup> shows that the effect of temperature in the two cases is opposite. In the case of primary infection of the seedling resulting from seed inoculation, high soil temperatures at the time of germination materially reduce the amount of disease, while in the case of secondary infection produced by spray inoculation high air temperatures favour the development of the disease, and low temperatures almost completely inhibit it. The optimum temperature for growth of the parasite in pure culture is about 27° C., while growth stops almost completely above 35° C. With the exception of one soil-temperature experiment this optimum does not agree with the temperature for maximum infection in either series of experiments. It seems possible that in the case of high soil temperature there is a direct effect on the parasite on the seed coat, preventing its multiplication and reducing its vitality before germination of the seed. This is supported by the fact that in the soil-temperature experiments, where the organism was introduced inside the seed coat, and was thus in contact with the embryo from the beginning, 100 per cent. infection resulted, even at a soil temperature of 40° C. In the case of the air-temperature experiments, where the parasite comes immediately into direct contact with the leaves, entrance through the stomata probably occurs within a very short time. It appears that the temperature relations of *B. malvacearum* within its host are different from those in pure culture or on some non-living surface such as the exterior of the seed, and that once penetration of the tissues has occurred a high temperature favours the development of the disease. The effect of temperature then becomes a problem of the balance between the physiological resistance of the tissues of the host, conditioned by the rate of growth and maturation and the biochemical reactions as influenced by the environment, and the activity of the parasite, itself in turn affected by the temperature. A clue to the nature of the biochemical factors that influence the development of the disease is given by a rough test carried out on the amount of reducing sugars present in the leaves at various temperatures. The work was done by Dr F. G. Gregory, to whom the author's thanks are due. The means of these estimations, which were done only on a small scale were: 23.5° C., 1.4 per cent.; 25° C., 2.6 per cent.; 31° C., 6.5 per cent.; 35° C., 6.9 per cent. It will be seen that there is a close correlation between these figures and the

figures for infection at the corresponding temperatures. Further work on this point is planned.

The other interesting point in these experiments is the effect of time of inoculation. Contrary to expectation the disease developed much more severely on leaves sprayed in the dark than on those in the light. Since entrance takes place through the stomata it would have seemed probable that infection would occur more readily in the light when the stomata are open. The explanation may be in the water relations of the host. During the light period it is reasonable to suppose that the rapid transpiration will result in a less amount of water either as liquid or vapour in the intercellular spaces and especially in the sub-stomatal chamber, whereas in the dark the amount of water present will be increased. The bacteria would then have greater opportunities for active motility and would enter the stomata more readily and in greater numbers during this period.

#### SUMMARY.

Experiments carried out in the Rothamsted control chambers on the influence of air temperature on the angular leaf-spot disease of cotton plants, resulting from spray inoculation of young plants, show that high air temperatures favour the development of the disease. Maximum infection occurs at an air temperature of 35–36° C. with decreasing incidence at progressively lower temperatures. At a constant air temperature of 39–40° C. cotton plants make no growth and eventually die.

Infection takes place more readily when the inoculation is carried out during the non-illuminated period.

The relation of these results to the experiments on the influence of soil temperature is discussed.

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- (3) — (1928). The influence of environmental conditions on the development of the angular leaf-spot diseases of cotton. *Ann. App. Biol.* xv, 333–41.
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**EXPLANATION OF PLATE XXXIX.**

Cotton leaves affected with angular leaf-spot disease, showing types of infection. Photographed by transmitted light.

Fig. 1. Type of infection characterised by discrete spots.

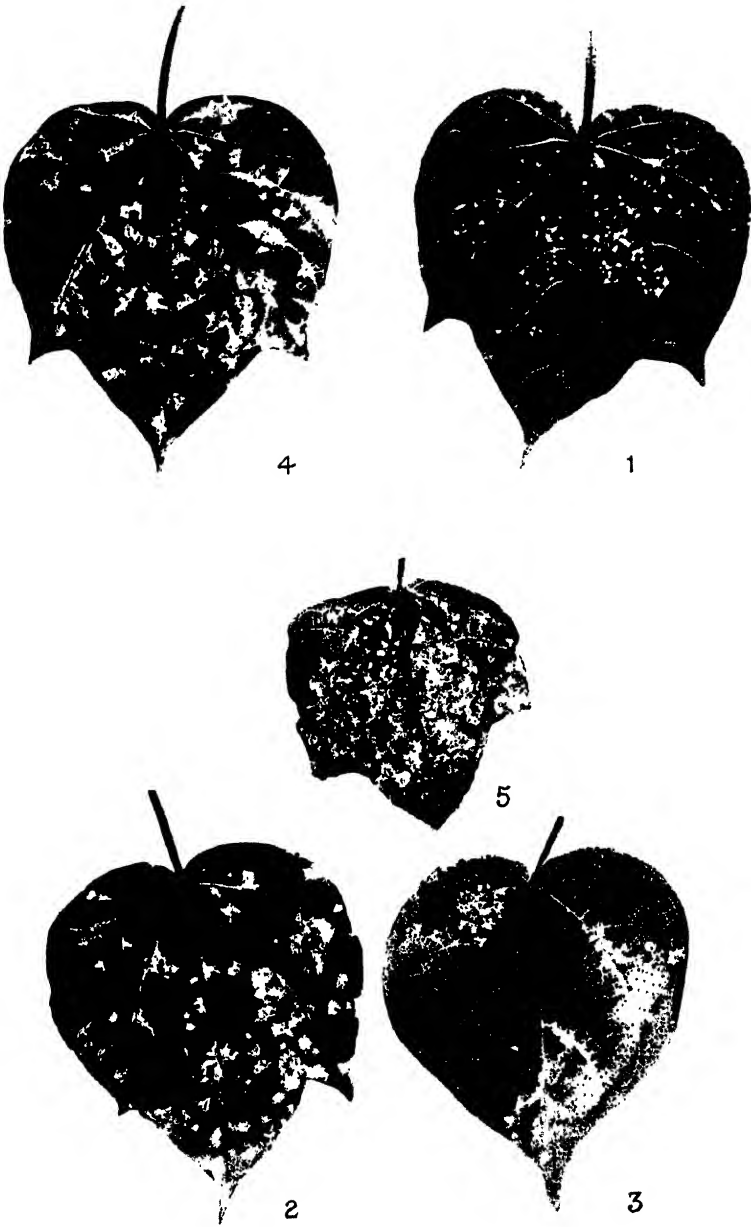
Fig. 2. Type of infection characterised by coalescence of spots.

Fig. 3. Type of infection characterised by extension along vein.

Fig. 4. Leaf showing "coalescence" and "extended" types of infection.

Fig. 5. Leaf showing severe infection described as "indefinite."

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STOUGHTON.—THE INFLUENCE OF ENVIRONMENTAL CONDITIONS ON THE DEVELOPMENT OF THE ANGULAR LEAF-SPOT DISEASE OF COTTON (pp. 524-534).





## INTRACELLULAR INCLUSIONS IN MOSAIC OF *SOLANUM NODIFLORUM*

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(With Plates XIII–XVI and 1 Text-figure.)

IN the course of a study of the aucuba or yellow mosaic of tomato, inoculation of the virus was made into an extended series of different host plants, and in the examination of these hosts search was made for the presence or absence of abnormal intracellular inclusions, and in particular for bodies of the amoeboid type called by Miss Goldstein X-bodies. The latter were readily found in every plant in which obvious symptoms developed, but were not found in hosts which did not take the disease. In the different hosts they presented minor differences of size, shape or apparent structure, but in all were sufficiently alike as to leave no doubt that they were in every case structures of the same nature. In one host, however, viz. *Solanum nodiflorum*, they were exceptionally conspicuous in the cells of the leaf-hairs, and as in this plant the X-bodies ordinarily occur unaccompanied by those other abnormal inclusions (striate material, crystal plates or packets, and the like) which, in many hosts, e.g. tobacco, crowd the cells and complicate the picture, it seemed to offer an unusually favourable opportunity for their more detailed examination. In the following pages are recorded some of the results of this investigation.

The virus of aucuba mosaic of tomato is closely akin to the classical virus of tobacco mosaic, from which it differs only in the greater brilliance of the symptoms it commonly produces (Henderson Smith<sup>(s)</sup>). In *Solanum nodiflorum* it causes a typical mosaic disease with the characteristic irregular mottling of the leaves (Plate XIII, fig. 1), which is readily transmitted by juice inoculation and in young actively growing plants regularly develops after a short incubation period of 5–12 days:

In this plant the hairs of the leaf are two- to four-celled structures, with rather rigid walls studded with numerous minute papillae (Plate XIII, fig. 2). They stand out stiffly from the epidermis, and along the margins of the leaf project in such a fashion and such numbers that

each individual hair can readily be examined under high powers of the microscope when mounted simply in water or other suitable medium. The cells of the normal hair show nothing but the nucleus, the peripheral cytoplasm and a varying number of strands of streaming protoplasm, with occasionally a few crystals and perhaps a little granular or amorphous matter of uncertain nature in the vacuole, which occupies the bulk of the cell. There are no chloroplasts. In the hairs of an infected leaf the cells contain in addition a large abnormal inclusion, the X-body (Plate XIV), which is never found in normal hairs, and has not been seen in the cells of any plant suffering from any pathological condition other than virus. There is often present also a large spike, which usually lies in the long axis of the cell, as if it were suspended in it, and appears to be crystalline. It can sometimes be seen to be made up of a bunch of hair-like crystals, especially distinguishable at the ends and is sometimes thicker in the middle, producing a slightly tapering appearance. There may be more than one such spike, and these may either be quite separate from one another or may approximate at one end so as to produce a radiating appearance (Plate XVI, fig. 1). They have no connection with the nucleus, and, although sometimes apparently related to the X-body, often are quite separate from it. Their appearance and position are often such as to suggest that they are crystalline formations either of or in cytoplasmic trabeculae.

Typically the X-bodies are roughly spherical (Plates XIV, XV), and usually there is a tendency to a rounding of the contours, whatever the shape. Often, however, they are quite irregular in outline and when in contact with the cell wall or septum are frequently flattened on that side. The smaller bodies, while tending also to rounding, are more usually irregular than the larger, and may appear, especially when lying along the cell wall, as elongated lumpy masses or as thin and rather flat. When very small, it is difficult to feel certain that they are really small sizes of the larger bodies. The size varies considerably. The large spherical bodies may reach  $30\mu$  in diameter; the small may go down to  $5\mu$  or less in their larger diameter; in five adjacent epidermal cells they measured 12.4, 12.2, 10.3, 8.5 and  $9.3\mu$  respectively. Even the larger bodies are partially translucent, and are tinted brown or pale yellow; the small bodies may show no colour, perhaps because there is not enough depth of substance to show it. In structure they are coarsely granular. In many cases, indeed, they look as if they were aggregations of smaller particles rather than truly homogeneous, and at the margins of the larger bodies, especially in fixed material, there may sometimes

be seen small projecting particles which confirm this impression (see Plate XIV, in the two terminal cells). But the appearance in this respect is not constant. Some bodies look much more homogeneous than others, *i.e.* are very finely granular. This is particularly well seen in the flat epidermal cells of the leaf, examined in the living state. There one may see in one cell a coarsely granular body, in which vacuolation can be made out, if at all, only with difficulty, and in an adjoining cell, whose margins are contiguous with the first, the body may be very finely granular and homogeneous, with conspicuous vacuoles (Plate XV). In the hair-cells, in the fresh preparation, vacuoles are not conspicuous in the coarsely granular bodies but careful focussing usually shows that they are present in its substance: and in fixed preparations, especially when stained with methylene blue, the vacuolation is usually quite evident. There may be only one or two vacuoles, but the number varies, as many as nine have been counted in one body (Plate XVI, fig. 3). In the coarsely granular type there is usually no sign of a bounding wall or membrane. Sometimes, however, such a membrane or skin does seem to cover part of the surface, and in the more homogeneous forms the suggestion of a skin over the whole or the greater part of the surface may be very strong. No membrane has been seen in *S. nodiflorum* so definite as that figured by Goldstein in tobacco (1), Fig. 3, p. 563).

In most cases the body is found in close contact with the nucleus, sometimes alongside it, often more or less enveloping it, never incorporated with it. In living cells the body has on more than one occasion been seen to impinge on the nucleus, producing in it a temporary indentation; and both nucleus and body can frequently be seen to move within the cell and quite independently, sometimes drawing apart, sometimes approaching one another, the movements of both being similar and without any suggestion of being autonomous. In fixed preparations the relative positions depend on what happened to be the situation at the time of fixation, and in many instances the two are widely separate. There is, however, as will be explained later, a tendency for the body to be formed, and to remain, near the nucleus, and close juxtaposition is the rule. Within the cell the body may occupy any position, but is usually towards one or other end, most frequently the proximal end. It is not unusual to find in adjacent cells of the same hair two bodies lying one on each side of the same septum. Actual continuity of the bodies has not been demonstrable in such cases. Similar appearances in other plants have been taken as evidence that X-bodies are capable of passing through cell-walls (Likhité<sup>(6)</sup>), and it may be that this is possible;

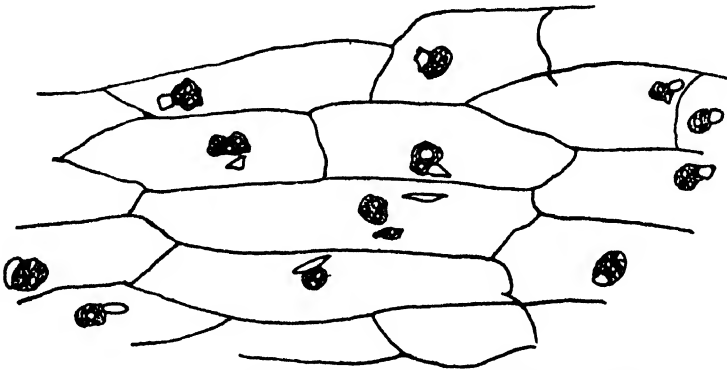
but other interpretations may obviously be given and no instance of such passage has been observed in the living tissues. All the cells of a hair may contain one or more bodies, or only one or two of the cells. In the latter case, it is most usual to find them present in the cells nearest the base and absent in the more distal cells, but rare instances have been seen in which the basal cell had none and the more distal cells contained them. The largest bodies are found in the basal cell and the cell next to it, those in the more distal cells being smaller. Since the morbid agent must pass up the hair from the base towards the tip, this might be interpreted as due to the longer duration of infection in the basal cells, but it seems to be generally true that the larger the cell, the larger the body it may contain, and this is in agreement with the experience of others (*e.g.* Kunkel in mosaic of maize<sup>(5)</sup>, Goldstein in mosaic of tobacco<sup>(1)</sup>).

In *S. nodiflorum* X-bodies show a pronounced tendency, not recorded for the similar bodies described in other plants, viz. a tendency to crystallisation. In large bodies this can often be clearly seen at the surfaces, where definite crystals project from the mass and are very visible at the margins (Plate XIII, fig. 3). But they are usually best seen in the elongated forms lying along the wall of the cell. There the mass can often be seen to be quite certainly partially crystallised, sometimes as fully formed crystals, sometimes as semi-crystalline forms with faces and angles on part only of their surface, such as are frequently seen in crystallising protein. The tendency is accentuated in certain fixing solutions, *e.g.* crystallisation is much more pronounced in material fixed in Bouin than in Carnoy; but it occurs in unfixed material and can be seen in cells where the continuance of protoplasmic streaming shows that the cell is still living. It is most evident in old leaves and in leaves in which infection has been of long duration.

The X-bodies may occur in any part of the leaf, *e.g.* the palisade or the cubical parenchyma cell: they are sometimes beautifully seen in the epidermal cells lying along the veins on the under-surface (Text-fig. 1). The most satisfactory method of demonstrating them in fixed material is first to stain the nuclei red by Feulgen's method, and then counter-stain with suitable dyes, such as methylene blue or aniline blue. The nucleus is thereby clearly differentiated from the body, even when partially embedded in it, and the vacuolation of the body is clearly brought out. As a rule they are present in largest numbers in tissues which are macroscopically chlorotic, but they are often to be seen in regions or even in leaves where there is no obvious chlorosis: in the

fern-leaf type of leaf in tomato, for example, they are conspicuous in the hairs (Plate XVI, fig. 4). In *S. nodiflorum*, in a leaf detached from the plant when it is beginning to show local signs of the disease, there may at first be no bodies present in the hairs of the yellow or any other part of the leaf, but later examinations of the same leaf (kept meantime with its petiole in water or nutrient solution) show the bodies appearing progressively in the hairs all round the leaf, not only in the yellow regions but in areas where there is no macroscopic evidence of disease. The relationship between symptoms and the development of X-bodies, however, requires, and will receive, further investigation.

A number of chemical and other tests have been made on these X-bodies in the attempt to get a clearer idea of their nature. These



Text-fig. 1. X-bodies in the epidermis above a vein; stained with Feulgen and methylene blue. The round or elongated unshaded bodies are the nuclei; the shaded vacuolate bodies are the X-bodies.

have been carried out mostly on the hairs *in situ* on a portion of leaf, because of the ease with which the processes can be watched under the microscope. Unfortunately the cuticle of the hairs is highly impervious. Reagents of most kinds enter the cells only slowly, and one usually finds that penetration takes place very unequally in different hairs, and even in different cells of the same hair, and may take place more rapidly through the base of the hair than directly through its walls. In most cases the results have been confirmed by treatment of sectioned material, both of the hairs and other tissues.

The X-bodies withstand boiling in distilled water for 20 minutes, and are not dissolved in alcohol of any strength, in acetone or in chloroform (though in the last they sometimes appear to lose compactness). Heated in a platinum crucible till charred black, the hairs retain their outline perfectly, but the bodies disappear. In 2.5*N* KOH or NaOH

solution, the bodies and the crystal spike dissolve rapidly, usually leaving no residue but occasionally a granular heap remains in the situation of the body. In stronger alkali, *e.g.* 5*n*, disappearance may be extremely rapid, both in fresh and fixed (Carnoy, Bouin) material. In strong sulphuric acid and concentrated hydrochloric acid, solution is also rapid; in 75 per cent. HCl, solution may take 15 to 20 minutes but is complete; in 50 per cent. HCl the bodies, even when partially crystallised, do not dissolve in 19 hours at room temperature, nor do they dissolve in strong acetic acid. In sodium hypochlorite they dissolve rapidly. No digestion was obtained with taka-diastrase, but probably penetration did not occur.

*Millon's reaction.* The bodies in fresh preparations turn brown to red-brown; after Carnoy fixation they were definitely brick-red, the colour being deepest where the body is thickest; and in cells where the body had developed definite crystals these also turn red; after Bouin fixation, bodies and crystal forms are red. The colour is intensified and its development accelerated by warming. In no case did the bodies dissolve. In the similar bodies of *S. nigrum* Millon gives a very pronounced red-brown colour.

*Raspail's reaction.* The leaf portions were left in a concentrated solution of saccharose for 3 hours or more, the solution then drained off and the preparation mounted in strong sulphuric acid. In fresh preparations, the bodies dissolve quickly, turning bright red as they do so, and the red colour diffuses through the cell from the body. After Carnoy fixation, the body may remain undissolved for a quarter of an hour or more, turning bright red; on solution of the body the colour remains localised. After Bouin fixation, no red colour was obtained, although solution was sometimes very slow. In sections of hairs, solution was almost immediate and no red colour was observed; in sections of palisade tissue, a brown-red colour was got before solution.

*Biuret reaction.* Fresh portions of leaf were placed for 3–4 hours in saturated solution of copper sulphate, washed well in distilled water, and mounted in 2.5*n* KOH. The bodies turned rose-pink in a few minutes and then yellow. After about 15 minutes dancing particles appear in the cell, the long crystal quickly disappears. Colourless, spherical, rather large droplets appear in the cell, which later become granular and are merged in the protoplasm which contracts from the walls. Then the body progressively breaks down into a mass of granules, which persist for some hours. After Carnoy fixation the whole body turns pink at once, and rapidly disappears. After Bouin fixation, it turns pink, and then yellow.

*Xanthoproteic reaction.* Tested on sections of palisade tissue, the body turns brown on addition of ammonia (not yellow).

*Prussian-blue reaction.* The leaf portion was kept in the ferrocyanide solution overnight, washed well in 60 per cent. alcohol, and then the ferric chloride added. Even after 2–3 hours, results are very irregular. In many hairs there is no staining at all, but in some the bodies are deep blue. It is the same after Carnoy fixation; after Bouin fixation, most bodies are a strong blue colour.

*Cinnamic aldehyde.* The preparations were left 48 hours in 1 or 5 per cent. solution in 60 per cent. alcohol, before the sulphuric acid was added. Bodies turn strong yellow with a tinge of red in both Carnoy and Bouin preparations; pale yellow in unfixed material. Anisaldehyde produced immediate reddening of the bodies. Salicylic aldehyde and vanillin gave no definite results, only a transitory colour developing. With these substances it is difficult to be certain that penetration occurs in the hairs. In sections of hairs, with strong sulphuric acid, vanillin produced a definite mauve-pink in the bodies; salicylic aldehyde gave no colour.

In view of the fact that potash starvation produces in the leaves of some plants a mottling not wholly unlike the mottle of mosaic, it was of interest to ascertain whether there was any indication of concentration of potassium in the X-bodies; and tests were made by the Molisch-MacCallum method (sodium cobalti-nitrite, followed by sulphide) on hairs that had been fixed in Carnoy. The cytoplasm throughout showed many very small black granules, and these were also present in the X-bodies in approximately the same proportion: the bodies did not as a whole turn black. It would seem, therefore, that potassium occurs equally in the cytoplasm and the bodies.

Iodine stains the bodies brown or yellow, not blue nor black. Sharlach R and Sudan III produced no red colour in them, even when penetration had certainly occurred. With osmic acid, the bodies turned brown but not black even after 24 hours in 3 per cent. solution. With material fixed in potassium bichromate, chromic acid and 2 per cent. osmic acid for 48 hours, then thoroughly washed in running water for 12 hours and, after repeated washings in distilled water, kept in 2 per cent. osmic acid for a week at 30–35° C., the bodies were a dark brown but did not have the characteristic black colour of fatty material.

When the living hair, mounted in water, is examined in polarised light, no pleochroism is observed in the cell walls, the crystal spike or the X-bodies. With crossed Nicols the cell walls and septa extinguish

on rotation, but neither the spike nor the bodies nor the crystals into which the body may have resolved appear distinctly at all. Sometimes a doubly refracting crystal is to be seen lying on the surface of the body, and sometimes one or two small crystals appear, embedded in the surface of the body. By ordinary light, the bodies, mounted unstained in Canada balsam or in xylol, are almost invisible; *i.e.* they have a refractive index of about 1.52.

From these various reactions it is evident that the X-bodies, like the striate material and crystal packets investigated by Klebahn<sup>(4)</sup> in other hosts, are proteid in nature. The fact, established by Holmes<sup>(3)</sup> in *Hippeastrum* mosaic, that they contain mitochondria suggests that they are protoplasmic in nature; but no nuclear material has been demonstrated in them in *S. nodiflorum* or in any other host. It is unnecessary here to recapitulate in detail the appearances which have led some observers to believe that they are independent living organisms. They are described in the papers by Kunkel<sup>(5)</sup>, Goldstein<sup>(1, 2)</sup>, Likhité<sup>(6)</sup> and others; and are discussed by the present writer in a paper shortly to be published in *Biological Reviews*. In the hairs of *S. nodiflorum* it has been found possible, by following up an observation made by Miss Sheffield in this laboratory, to watch in individual living cells the development of the X-body from its early beginnings to complete formation<sup>1</sup>. It appears that soon after the virus enters the cell tiny plastic particles appear in the circulating cytoplasm and are carried along in its stream. These particles gradually increase in size, and tend to pause in their course at the junctions or anastomoses of the cytoplasmic strands, before adaptation of the shape of the particles and adjustment of the strands allow them to proceed. During such a pause one particle may be joined by another, and when movement is resumed, the two may either separate or may go on as one united mass. In this way larger and larger masses are built up, until they can be recognised as undeniable X-bodies. There may be several such bodies in the one cell, arising independently and remaining distinct, or they may unite to form one single X-body. The composite bodies may again break apart into two or more smaller bodies, and in this way appearances are presented which might suggest fission; and not infrequently, when a smaller mass joins, or breaks away from, a larger one, appearances occur which simulate pseudopodia and have been so interpreted. There is, however, no division in the sense of multiplication, and the separated masses may

<sup>1</sup> A preliminary account of this work has already been published (Sheffield and Henderson Smith (7)), and a more complete account will be given later.



again unite. The movement is always passive: there is no suggestion of autonomous movement. Both the nucleus and the body may continue to move similarly and independently of one another, even in cells which contain only one large X-body where the process of formation is apparently complete.

This mode of formation accounts for the coarse granularity already described as frequently observed in the bodies of the hairs or epidermis. They are actually aggregates<sup>1</sup> in their earlier stages, but when the aggregated particles have been in contact for a time, they seem to fuse together into a more homogeneous mass, in which vacuolation is distinct. It explains also the tendency of the bodies to be found in close association with the nucleus, because it is there that the cytoplasmic strands meet, and it is in such situations that the bodies tend to form. The nature of the small particles is still undetermined. They give the impression of being foci where the cytoplasm has condensed or consolidated; and, if so, their protein nature and mitochondrial content are intelligible. The body itself should be regarded as a product of the reaction produced in the cell cytoplasm, and it may be that each of the tiny particles is evidence of a local reaction to an ultra-microscopic particulate virus embedded in its substance.

Grateful acknowledgment is made to Dr Margaret Madge for repeated assistance, especially in the cutting of many sections, and the carrying-out on them of various chemical reactions; also to Miss M. M. Browne for the skill and care given to the many plants used in this investigation.

#### SUMMARY.

A description is given of the intracellular inclusions found in *S. nodiflorum* after inoculation with the virus of the yellow (aucuba) mosaic of tomato. In this plant these inclusions are unusually conspicuous and can be observed in the living cell with exceptional ease. They are of two main types, crystalline spikes and amoeboid bodies, the latter corresponding to the X-bodies found in other plants. A detailed account is given of their appearance, structure, position in the cells, relation to the nucleus etc. The X-bodies may be either coarsely granular, looking like aggregates of particles, or more homogeneous; and they have a pronounced tendency to crystallise into the forms commonly seen in protein crystals. They are vacuolate, and occur in all the tissues of the leaf.

<sup>1</sup> When a portion of leaf containing well-formed bodies in every hair is mounted in distilled water and evaporation prevented, the bodies may break down again and disappear from the cells within 48 hours.

They give the usual tests for protein (Millon, Biuret, etc.) and are not of fatty nature. Their formation has been followed in individual living cells from the very early stages to their complete development. The mode of formation, viz. by the aggregation of small particles, which are carried in the protoplasmic streaming, is shown to account for the appearances which have led various observers to conclude that they are living organisms or parasites, a conclusion for which no evidence has been found in the present investigation.

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## DESCRIPTION OF PLATES XIII—XVI

## PLATE XIII.

- Fig. 1. Leaf of *S. nodiflorum*, inoculated with aucuba mosaic. Length of leaf: 4 inches.  
 Fig. 2. Hair of *S. nodiflorum*: normal. Carnoy fixation; unstained;  $\times 310$ .  
 Fig. 3. Crystallising X-body in leaf-hair. Bouin fixation; unstained;  $\times 450$ . Nucleus partly seen at left upper edge of mass of crystals.

## PLATE XIV.

Hair showing X-bodies. Carnoy; unstained;  $\times 430$ . Note the granular appearance; and in the two terminal cells the projecting particles. In the third cell the nucleus is visible at the upper edge of the body.

## PLATE XV.

Epidermis. Fixed in Carnoy; stained with Feulgen and methylene blue;  $\times 820$ . The small dark bodies are the nuclei, the larger bodies the X-bodies. Above the stoma is a cell where nucleus and X-body are not in juxtaposition.

## PLATE XVI.

- Fig. 1. Hair-cell showing two radiating crystal spikes. Fresh preparation, mounted in water; unstained. Nucleus visible at upper edge of body.  
 Fig. 2. Section of palisade tissue. Carnoy; Hoidenhain and anilin safranin.  
 Fig. 3. X-body in epidermis of leaf. Carnoy; Feulgen and methylene blue;  $\times 1750$ . The smaller dark body is the nucleus.  
 Fig. 4. Glandular hair from fern-leaf in tomato. Fresh preparation; unstained. Note the separation of nucleus and X-body in the cell next the base-cell, and their juxtaposition in the cell above.

(Received November 1st, 1929.)



Fig. 1.



Fig. 2.

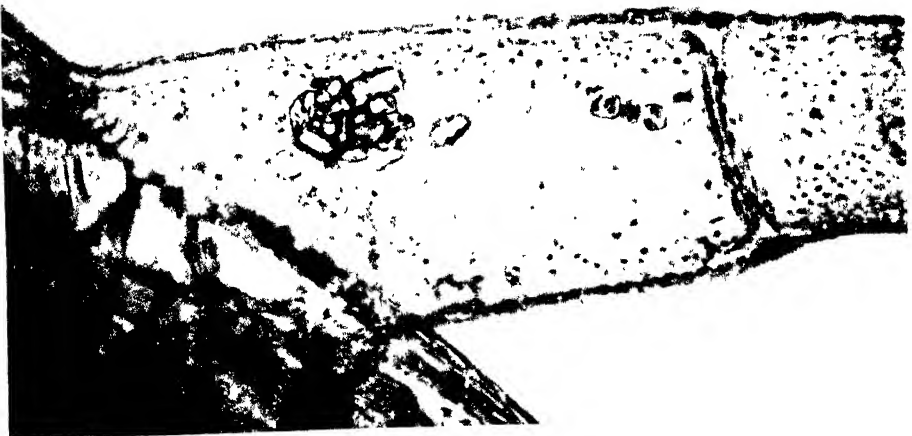
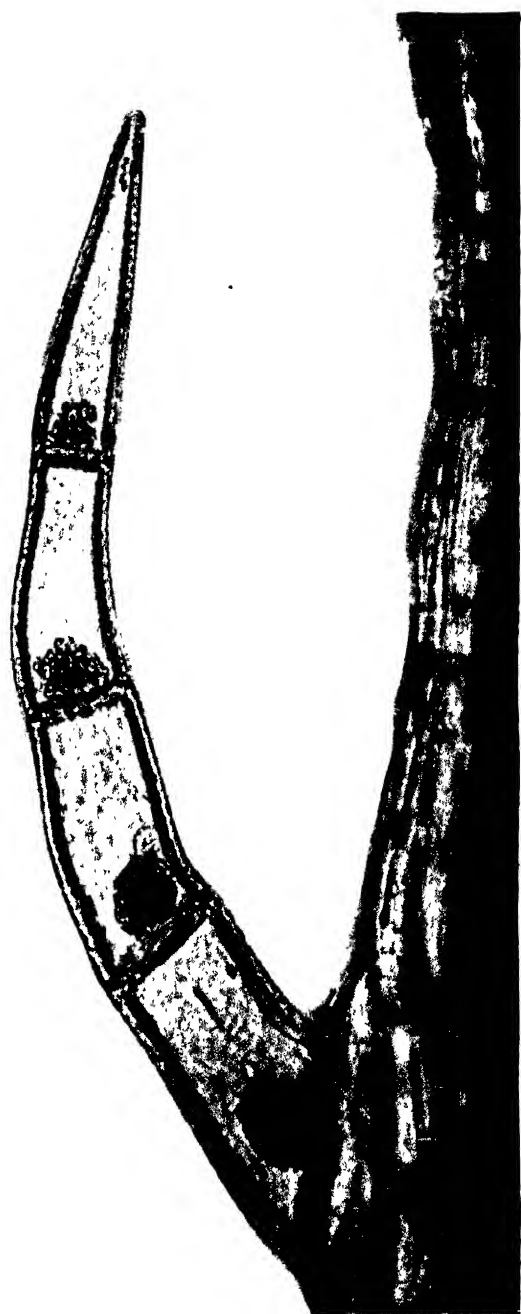


Fig. 3.













## STREAK—A VIRUS DISEASE OF TOMATOES

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(With 2 Diagrams in the Text.)

### INTRODUCTION.

STREAK disease of tomatoes has for many years been recognised as one of the most severe diseases to which this plant is susceptible. The literature contains, under various names, lengthy descriptions of symptoms of diseases apparently identical with streak. The terms stripe, black stripe, winter blight, severe mosaic, and probably spotted wilt, all appear to have reference to this same disease.

Tomato streak occurs frequently in commercial glasshouses in the south of England, where tomatoes are forced for an early market. It agrees in its symptoms with the description given by Vanterpool<sup>(13)</sup>, Gardner and Kendrick<sup>(7)</sup>, Brittlebank<sup>(5)</sup>, and others, and is probably identical with tomato stripe (Bewley<sup>(2)</sup>). Many tomato plants attacked by this disease show leaf mosaic symptoms only; others show, in addition to mosaic, large necrotic areas on the leaves accompanied by longitudinal, dark, necrotic lesions on the stems and petioles, whilst on other plants similar necrotic areas may be found without any mosaic being present. Frequently, however, a plant, which at first shows mosaic only or necrosis only, subsequently develops the other type of symptom also.

The evidence for the statement that this disease is either bacterial or malnutritional in origin is inconclusive, and it is now generally recognised to be a virus disease. However, there seems to be little agreement among investigators as to the identity of the virus causing streak. No less than three theories are supported: (a) that this disease is due to the virus of tomato or tobacco mosaic—tobacco virus I (Johnson<sup>(11)</sup>), which under certain environmental conditions takes on increased virulence and causes necrosis in addition to mosaic; (b) that two components are concerned in the virus of streak, one producing the mosaic, and the other, the necrotic streaks (cf. Boning<sup>(4)</sup>); (c) that streak is the result of a mixed infection by the viruses of tobacco mosaic, and potato mosaic

(cf. Dickson<sup>(6)</sup> and Vanterpool<sup>(13)</sup>). The disease resulting from the combination of the two latter viruses is admittedly indistinguishable from glasshouse streak, and is termed "experimental streak" in the work to be described.

Blood<sup>(3)</sup> claims to have produced a streak disease in tomatoes by inoculation with "a disturbing principle from apparently healthy potatoes in combination with tomato mosaic virus." However, in view of the failure of Henderson Smith<sup>(9)</sup> in tomato and of Kenneth Smith<sup>(12)</sup> in tobacco to produce any disease by inocula from absolutely healthy potatoes, it is probable that the potatoes employed by Blood contained a mosaic which was masked.

Berkeley<sup>(1)</sup>, on the other hand, states that "it is not necessary to have a combination of viruses in order to produce streak, since the juice of healthy potatoes in itself is sufficient for this purpose." However, in this conclusion, this worker appears to have confused with streak the disease which is produced in tomatoes by the juice of apparently healthy potatoes (Johnson<sup>(10)</sup>) or of potatoes infected with mosaic (Henderson Smith<sup>(9)</sup>).

#### EXPERIMENTAL.

This paper is concerned with a comparison of glasshouse streak and experimental streak. The source of glasshouse streak was a commercial glasshouse in which the incidence and spread of the disease among tomato plants was watched during several months. Separate samples were taken from plants showing the three forms of the disease, viz. those showing mosaic only, those showing necrosis only, and those showing mosaic and necrosis on the same plant. The virus of tobacco mosaic tobacco virus I<sup>(11)</sup> was furnished by Dr Grainger of Leeds University, who obtained it from Dr Johnson of Wisconsin. The virus of potato mosaic was derived by Dr Henderson Smith from mosaic Up-to-Date potatoes, the leaves of which when inoculated into tomato produced a characteristic disease<sup>(8)</sup>.

#### METHODS.

*Preparation of plant extracts.* In all cases this was done by grinding the minced leaves of diseased plants with water in a mortar, 3 c.c. of water being added for each gram of leaf tissue. For immediate transmission of a disease from one series of plants to another, inoculation was made with this pulp, but when a stock of sterile infective juice was required, the method of filtration described by Henderson Smith<sup>(8)</sup> was

adopted. The filter cylinder used preparatory to the Pasteur Chamberland filters was composed of alternate layers of sand and macerated ashless filter paper tightly packed. The final dilution of plant juice in water was not greater than 1 in 9 in the case of tomato, and 1 in 5 or 6 for tobacco extracts. To prevent evaporation, paraffin wax was added to the cotton wool plugs of the tubes containing the extracts, and in this way a bacteriologically sterile stock can be kept indefinitely.

*Methods of inoculation.* Inoculations were usually made by pricking about forty times with a needle, three or four of the youngest leaves of a young vigorously growing, healthy plant. For this purpose the leaf was placed in the inoculum lying on the flat wooden label to be used for that particular plant. In some cases, the plants were inoculated at the base of the stem below the first leaves, by three or four longitudinal incisions in the succulent stem, and into these incisions the extract was inserted. Both methods gave positive results with equal regularity, although the incubation period following inoculation by the second method was invariably at least one day longer.

When individual plants were inoculated with two viruses, as, for example, in the production of experimental streak, either equal volumes of the extracts containing each different virus were mixed together and used as one, or both extracts were inoculated separately into two leaves. These methods were equally effective.

Precautions were taken to protect the experimental plants from secondary infection.

#### DETAILS OF EXPERIMENTS.

The first experiment was carried out with the original diseased tomato plants from the commercial glasshouse. From the difference in appearance of the two types of symptoms (the coarse mottle or mosaic and the necrosis of the leaves and stems) streak, as has been mentioned above, has been regarded as being due to two factors. This experiment was designed to show whether this distinction of symptoms remained constant, and to ascertain the effect of filtration on the infectivity of the inoculum. To this end, filtered and unfiltered extracts of plants showing each type of symptom independently, and of plants showing both forms together were inoculated into tomato plants (variety Kondine Red) and tobacco plants (variety White Burley), seven plants being used in each series. As a control, the original plants (two in each case) were grown in the experimental glasshouse, and in addition two cuttings were made from each of them. One of the two plants showing mosaic

only developed in course of time necrotic spots on the upper leaves and typical lesions on the stem, while the leaves from both of its cuttings developed large necrotic areas. Both the cuttings and also the new apical and lateral shoots on both plants formerly showing streak only developed conspicuous mosaic symptoms.

Table I.

Inoculum		Tomato. Symptoms produced		Tobacco. Symptoms produced	
		Mosaic only	Mosaic and streak	Mosaic only	Mosaic and streak
Mosaic only:	Filtered	5	2	2	5 (1 killed)
	Unfiltered	6	1	2	5
Streak only:	Filtered	7	0	3	4 (1 killed)
	Unfiltered	5	2	2	5 (2 killed)
Mosaic and streak:	Filtered	6	1	3	4 (1 killed)
	Unfiltered	4	3	4	3

The results given in Table I show that, judged by the symptoms produced on sub-inoculation, no difference could be detected between the different inocula. Glasshouse streak, no matter how virulent it appeared to be in the inoculum, did not produce pure streak (*i.e.* necrotic) symptoms when inoculated into healthy plants, nor does pure mosaic in the inoculum mean that streak may not result on sub-inoculation. Moreover, filtration through Pasteur Chamberland L. 1 and L. 3 candles does not reduce the infectivity of the disease.

*Resistance to heat and alcohol.* It was thought that the mosaic and the streak factors, if these be distinct, might be differentiated by their reactions to various degrees of heat and alcohol. Accordingly, filtered extracts from single tomato plants showing both coarse mosaic and streak on the one plant were (1) heated for 10 minutes at 60°, 70°, 80°, 85° and 90° C. and immediately afterwards cooled under running water, and (2) treated for 1 hour at concentrations of 60, 70, 80 and 90 per cent. alcohol. The precipitate formed by the action of the alcohol on the plant juice was separated from the supernatant liquid by centrifuging, and then shaken up in distilled water, making the final volume up to the original volume of plant juice. Subsequent inoculations showed that the precipitate contained the virus, and the supernatant liquid did not.

The virus was inactivated by 90° C. for 10 minutes, but retained its virulence at 85° C., and also after treatment with 90 per cent. alcohol for 1 hour. This experiment was repeated with extract from tobacco inoculated with glasshouse streak with essentially the same results.

Although the proportion of streaked plants was low, mosaic and streak symptoms appeared with equal regularity over the same range of heat and alcohol concentration.

Table II.

(a) *Effect of heat on the infectivity of glasshouse streak.*

Inoculum	10 mins. at	No. of plants	No. positive	Symptoms		
Glasshouse streak show-	60° C.	7	7	6 mosaic only, 1 mosaic and streak		
ing both mosaic and	70	7	7	4	"	3
streak	80	7	7	5	"	2
	85	7	7	5	"	2
	90	7	0	Nil		

(b) *Effect of alcohol on the infectivity of glasshouse streak.*

Inoculum	Alcohol strength %	No. of plants	No. positive	Symptoms		
Glasshouse streak show-	60	7	7	5 mosaic only, 2 mosaic and streak		
ing both mosaic and	70	7	7	4	"	3
streak	80	7	7	5	"	2
	90	7	7	6	"	1
Control untreated	—	7	7	5	"	2

Excessive nitrogen manuring and forcing of young plants is reported by growers and others to induce the formation of streak, and these treatments were tried on the plants used in the above experiment. At the end of the fourth week excessive nitrogen (1.13 gm. of sodium nitrate per 6-inch pot) was added to three plants in each series, while the tops of the other three were forced by cutting away all axillary buds and shoots. However, only four more plants out of sixty so treated developed streak after this date, and they are not included in the table above as they developed it after so long an interval that secondary infection could not certainly be excluded.

In all experimental plants showing mosaic only, the close similarity of the symptoms to those of tobacco mosaic in tomato was particularly noticeable, and the comparison may be extended when one examines the resistance of tobacco virus I to heat and alcohol (see Table III). It will be seen that this virus retains its activity entirely after treatment with 90 per cent. alcohol and almost entirely after heating for 10 minutes at 85° C., results which agree with those of others. Tobacco mosaic, therefore, shows resistance of the same order as that of glasshouse streak to alcohol, and heat.

Table III.

*(a) Effect of heat on the infectivity of tobacco virus I.*

10 mins. at	No. of plants	No. positive	Symptoms
60° C.	7	7	Mosaic only
70	7	7	"
80	7	7	"
85	7	6	"
90	7	0	Nil

*(b) Effect of alcohol on the infectivity of tobacco virus I.*

1 hour of	No. of plants	No. positive	Symptoms
70 %	7	7	Mosaic only
80	7	7	"
90	7	7	"
Control untreated	7	7	"

*Combined inoculations.* From the above comparisons it seemed probable that glasshouse streak would act in the same way as tobacco mosaic when combined with potato mosaic—that is, produce experimental streak. Potato mosaic was accordingly combined with tobacco mosaic in one series of inoculations, the combination producing a disease here referred to as experimental streak 1; and in another series it was combined with glasshouse streak, and produced a disease, here called experimental streak 2. These forms of streak were identical in the length of incubation period, as well as in the manner of appearance and intensity of symptoms. On the other hand, the combination of tobacco mosaic and glasshouse streak produced the symptoms of tobacco mosaic only, until on the 22nd day one of the seven inoculated plants developed necrotic leaf spots without stem lesions—*i.e.* this combination did not produce streak.

*Resistance to ageing in vitro.* Filtered extracts of glasshouse streak, tobacco mosaic, potato mosaic and experimental streaks 1 and 2 were kept bacteriologically sterile in sealed test tubes in a cupboard, in subdued light, and after varying lengths of time their virulence was tested by inoculation into healthy tomatoes.

From Table IV it is seen that the virus of glasshouse streak in tomato or tobacco extract retains its virulence stored *in vitro* for at least 16 months, while tobacco virus I in tomato extract is virulent after 12 months under similar conditions. Dickson (8) records that expressed juice of mosaic diseased tobacco plants, unfiltered and protected from contamination by a layer of toluene, was still virulent after storing for 5 years. On the other hand, potato mosaic appears to have lost its

Table IV.

(a) *Single viruses.*

Virus	Age in months	No. of plants	No. positive	Symptoms	
Glasshouse streak in tomato extract	6	7	7	*7 mosaic only	
	9	6	6	5	" 1 mosaic and streak
	12	6	6	4	" 2 "
	16	6	6	*6	" "
Glasshouse streak in tobacco extract	6	7	7	*7	"
	9	6	6	3	" 3 "
	12	6	6	2	" 4 "
	16	6	6	*6	"
Tobacco mosaic in tomato extract	6	6	6	6 mosaic	
	12	6	6	6	"
Potato mosaic in tomato extract	3	6	6	6	fine spot necrosis
	5	6	6	6	"
	6	6	0	Nil	
	9	6	0	Nil	
Potato mosaic in tobacco extract	9	6	0	Nil	

\* In each of these cases, the juice was tested in late autumn when growth of the plants is very slow and glasshouse streak symptoms are not usually obtained in England.

(b) *Viruses in combination.*

Virus	Age in months	No. of plants	No. positive	Symptoms	
Experimental streak 1 in tomato extract	3	7	7	7 experimental streak	
	5	7	7	7	"
	6	6	6	6 mosaic only	
	12	6	6	6	"
Experimental streak 2 in tomato extract	5	7	7	6 experimental streak, 1 mosaic only	
	6	12	12	1	" 11 "
	8	6	6	6 mosaic only	
	12	6	6	6	"

power of infection after 6 months' storage *in vitro* in tomato or tobacco extract, since it no longer produces a disease in tomatoes. According to Henderson Smith<sup>(9)</sup>, this mosaic virus from Up-to-Date potatoes was inactive after 12 weeks in filtered tomato juice, while the virus of mosaic in the variety Majestic remains infective for at least 5½ months. Extracts from plants which have been inoculated with two viruses, viz. experimental streak 1 or 2, have power to reproduce these diseases entirely for only 5 to 6 months, after which time it is assumed that the potato virus has lost its virulence, for the resulting symptoms are those of tobacco mosaic, or the mosaic of glasshouse streak only. After 12 months—no longer period has yet been tried—these symptoms appear regularly.

Resistance to ageing *in vitro* is a character which is preserved by individual viruses alone, or when in combination in plant extracts. This property is useful along with resistance to heat and alcohol in separating a single virus from a mixture. It is interesting to note that the virus of potato mosaic is less resistant than tobacco virus I or glasshouse streak to all three of these treatments.

*Host range.* Use of a number of different hosts as a means of separating and identifying viruses was made with special regard to two characters, the symptoms produced by each virus, and the length of time between inoculation and appearance of these symptoms, that is the incubation period of the virus in the host. The host plants tested were *Nicotiana tabacum* (var. White Burley), *N. affinis*, *Lycopersicum esculentum* (var. Kondine Red), *Solanum nigrum*, *S. dulcamara*, *S. villosum*, *S. nodiflorum*, *Nicandra physaloides*, *Petunia violacea*, *Hyoscyamus niger*, *Datura stramonium* and *Cucumis sativus*. Glasshouse streak and tobacco mosaic were the inocula, six plants being used in each series. All the above-mentioned plants, except *Datura stramonium* and Cucumber, showed pronounced leaf mosaic often with some distortion due to the irregular raised dark green areas, symptoms characteristic of tobacco mosaic. Not only did the same plants take both these viruses but they showed identical symptoms developing after the same incubation period.

Cucumber showed no symptoms at all with either virus, while *Datura stramonium* showed only frequent, dark brown, stem lesions below the nodes of the lower leaves, and on two plants slight necrosis of the leaf at the site of inoculation. Bewley(2), working with mosaic disease of tomato in *Datura stramonium*, says that no mosaic symptoms developed, but that all the plants showed "Stripe," a term which appears to be synonymous with "Streak." It is possible that he referred to these brown stem lesions also.

On the other hand, *Datura stramonium* has been shown to take potato mosaic, giving pronounced leaf symptoms (Henderson Smith(9)). This plant therefore suggested a means of separating potato mosaic from its combination in experimental streak 1 and 2. Also, if glasshouse streak contains this factor, *Datura stramonium* would be expected to show it.

Potato mosaic was transmitted to *Datura* in each case and from *Datura* back to tomato, but this did not occur with glasshouse streak. In experimental streak 1 and 2, the potato mosaic component appeared to be responsible for the leaf symptoms in *Datura*, but the dark stem lesions produced by tobacco mosaic and glasshouse streak were also



formed; only the potato mosaic virus, however, was transmitted back to tomato. It appears from these results that potato mosaic was not present in the glasshouse streak used.

Table V.

(a) *Tomato—Datura inoculations.*

Host	Inoculum	No. of plants	No. positive	Symptoms
<i>Datura stramonium</i>	Glasshouse streak	8	8	Brown stem lesions; plant No. 2 showed necrosis of veins of an inoculated leaf
	Potato mosaic	8	8	Fine mottle on leaves, fine rings formed and frequent small necrotic spots
	Experimental streak 1	8	8	Fine mottle on leaves, with brown stem lesions in all plants
	Experimental streak 2	8	8	Fine mottle on leaves in all and brown stem lesions in 7 plants

(b) *Datura—Tomato inoculations.*

Leaves of plants Nos. 1 and 2 from each of the above series were then inoculated back into tomato, 3 weeks later.

Host	Inoculum	No. of plants	No. positive	Symptoms
Tomato	Glasshouse streak	8	*1	Typical coarse mosaic
	Potato mosaic	8	8	Necrotic leaf spots and fine mottling
	Experimental streak 1	8	8	" "
	Experimental streak 2	8	8	" "

\* This plant was inoculated with material which contained the necrosed area of plant No. 2 noted in the corresponding series above; it is probable that in this necrotic area the glasshouse streak of the original inoculum remained virulent and produced the mosaic in this single plant when reinoculated into tomato.

## DISCUSSION.

Many workers dealing with streak disease of tomatoes have experienced difficulty in reproducing streak symptoms regularly, by artificial means of inoculation, a difficulty which was not overcome by the writer. On the other hand, experimental streak can invariably be reproduced. In the following diagrams we have this difference shown clearly.

Six plants were inoculated with glasshouse streak (Diagram 1), and six with experimental streak (Diagram 2). In both cases the inoculum had been stored *in vitro* for 6 months and in the latter—experimental

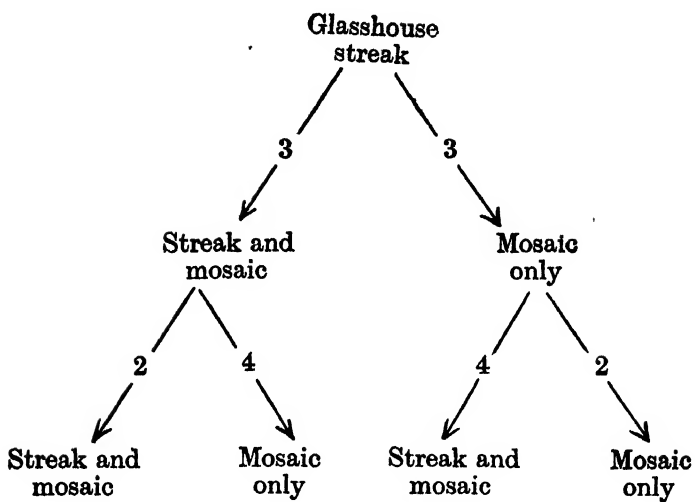


Diagram 1.

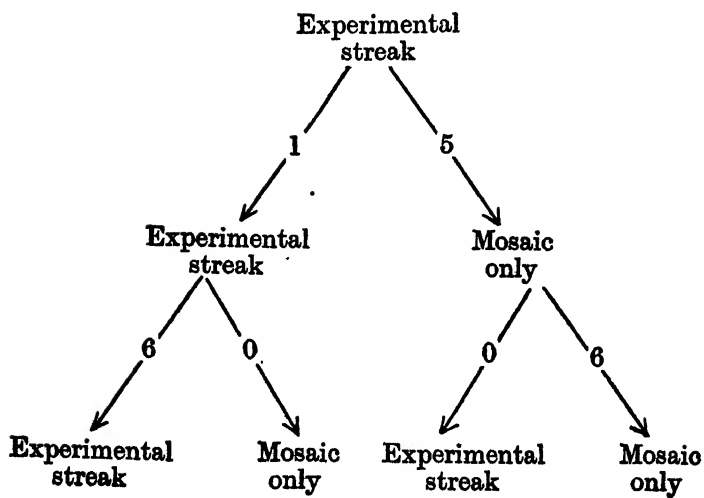


Diagram 2.

streak—the infectivity of the potato mosaic factor was so reduced that only one out of six plants developed experimental streak, but successive sub-inoculations from the other five plants produced the coarse mosaic of tobacco virus I only, as was to be expected. With glasshouse streak, streak and mosaic symptoms appear irrespective of the symptoms shown by the plants from which the inoculum was derived.

In experimental streak, the virus of potato mosaic appears to be the factor responsible for the necrosis of the plant, for tobacco virus I alone rarely, if ever, produces necrosis in tomato. It is difficult to understand why the addition of this potato virus to tobacco virus I should cause such a virulent disease which may practically kill its host.

No indication that glasshouse streak contains the virus of potato mosaic has been found, and necrotic lesions have occurred after juice containing the virus of glasshouse streak has been subjected to treatments with alcohol and heat and storage *in vitro* calculated normally to destroy the infectivity of potato mosaic.

The complete agreement in symptoms, resistance to alcohol, heat and ageing *in vitro*, host range and general characters which has been shown in this paper suggests that the virus of tobacco mosaic and glasshouse streak are probably one and the same. As glasshouse streak has been shown not to contain potato mosaic, necrosis must be due to another factor, perhaps connected with the reaction of the host and its physiological condition at the time of inoculation or, as generally assumed, necrosis only occurs under certain experimental conditions which have still to be defined.

#### SUMMARY.

A comparison of streak disease of tomatoes, derived from commercial glasshouses, and experimental streak produced by combined inoculation of the viruses of potato mosaic and tobacco mosaic, is given in detail.

The characters employed in comparison are the host range of each virus and its resistance to various temperatures, to different concentrations of alcohol, and to ageing *in vitro*.

Glasshouse streak and tobacco mosaic show an equal resistance to alcohol, heat and ageing *in vitro*, and have, in addition, an identical host range. Treatment for 1 hour with 90 per cent. alcohol and for 10 minutes at 85° C. did not destroy the infectivity of either of these viruses.

Glasshouse streak is shown not to contain the virus of potato mosaic, but is of itself able to produce necrosis in tomatoes without the partici-

pation of potato mosaic. The factors underlying this have not been determined.

It is concluded that tobacco mosaic and the mosaic of glasshouse streak are probably identical, and that much of the streak occurring in glasshouses is due to a single virus, and not a mixed infection of this with potato mosaic.

The author wishes to express her indebtedness to Sir John Russell, F.R.S., Director of the Rothamsted Experimental Station, for putting at her disposal the facilities of the Station, and to Dr W. B. Brierley, Head of the Department of Mycology, in which this work was carried out, the author at the time being the holder of a research studentship awarded by the Australian Council of Scientific and Industrial Research.

The assistance given by Miss M. M. Browne in the growth and care of the many experimental plants required in this work is gratefully acknowledged.

The thanks of the author are especially due to Dr J. Henderson Smith, whose ready advice and assistance throughout have been invaluable.

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## NOTES ON THE CULTURING OF INSECTS FOR VIRUS WORK

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(With Plate XXXVI and 3 Text-figures.)

### I. THE USE OF CELLOPHANE FOR BREEDING CAGES.

NOT the least important of the difficulties faced by the breeders of insects for virus experiments is the necessity for maintaining stocks unmixed and free from infection. As a general rule it is impossible to keep each set of insects in a separate glasshouse chamber, and, in any case, it is obvious that no chamber is "insect proof" if people pass in and out. The insects have therefore to be kept in cages which must possess certain characteristics, *i.e.* they must be portable, airy, light (transparent) and minutely insect proof, as many of the suspect insect vectors are very small and ubiquitous, *e.g.* thrips, white fly, red spider.

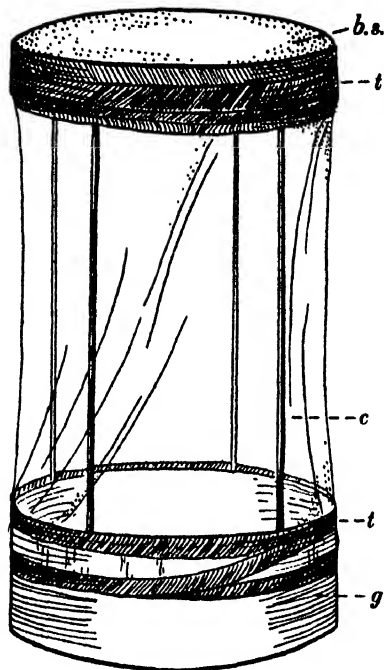
The materials most generally used are muslin, glass, or a combination of both, but these have many disadvantages. Muslin is not easy to handle satisfactorily and harbours eggs and small insects in its folds. If it is fine enough to keep out very minute pests it cuts off a great deal of light, a failing particularly noticeable in the winter months, and even coarse muslin has to be removed for observation of the culture. Glass is fragile; for large cages it is unwieldy (and also expensive), and for small ones causes a high percentage of humidity which is bad for many kinds of cultures, especially over long periods.

Text-fig. 1 shows a type of cage which overcomes some of these difficulties. Its basis is the usual type of light metal frame, consisting of four upright wires supporting two galvanised iron bands, 1½ inches and 3 inches deep respectively. A useful size is 14 inches height by 7 inches diameter, but the dimensions can be modified in accordance with the purpose of the cage. The walls are of cellophane<sup>1</sup> and it is roofed by fine bolting silk<sup>2</sup>. Cellophane is a material which is becoming invaluable to the entomologist. It was first brought to my notice by Dr A. D. Imms,

<sup>1</sup> The Cellophane Co., 7, 8 and 9 Bird Street, London.

<sup>2</sup> Dufour Bolting Silk, Henry Simon, Ltd., 20 Mount Street.

who had observed its use in America. Dr Imms has also been helpful with other suggestions, including information on the subject of bolting silk. Cellophane is a cellulose composition, light, durable, perfectly transparent and non-porous, though it allows the diffusion of water-vapour and other gases<sup>(3)</sup>. Smith<sup>(3)</sup> gives a description of its behaviour with regard to water-vapour, and mentions its permeability to ultra-violet light which is said to be the same as that of quartz glass.



Text-fig. 1. Cellophane-covered cage for culturing aphides on small cabbage plants.  
*b.s.* Bolting silk cover. *c.* Cellophane. *g.* Galvanised iron base. *t.* Insulating tape binding.

Incidentally, as a material it is decidedly cheaper than any other, except perhaps the very coarsest of cheese-cloths and muslins.

These cages are easy to handle and occupy a minimum of space in the glasshouse. Perhaps the best method of construction is as follows—starting from the finished wire frame which can be made by the local ironmonger or tin-smith, the metal bands are smeared with a waterproof cement, which can be made by dissolving cellulose acetate in ethyl acetate: similar preparations are sold in tubes like seccotine<sup>1</sup>. A sheet of

<sup>1</sup> "Pear Drop" waterproof and household cement, the Turnbridge M.F.G. and Supply Co., The Nurseries, Tangleway Road, Tooting, S.W.

cellophane of suitable size is quickly and not too tightly wrapped round, leaving plenty of margin at the top and the bottom. If it is too tight it is liable to split, as cellophane shrinks when damp. The free edges are then joined by a liberal application of cement, and a circle of bolting silk is gummed round the edges and stretched over the top by an elastic band. It is then finished by a tight strapping of ordinary 1-inch insulating tape which covers the free edges of the material, and is arranged so as to protect the cellophane where it is drawn over the sharp metal edge. As the insulating tape is only intended to stick to itself, the strapping must be made to overlap. If, as shown in Text-fig. 1, only two bands are used at the base of the cage, the inner free end should be well covered and the strapping should be finished on the upper edge of the metal so that the outer free end is above the level of the water in which the cage stands. It is advisable to cement down the outer edge, as the rubber solution tends to rot.

The cages stand in damp sand (for aphid) or water (for thrips as they might pupate in damp sand) in any flat receptacle of convenient size. Large earthenware plant-pot saucers are useful for holding the smaller cages. The whole can easily be removed from the chamber to be opened, but it is not necessary to open the cages for general observation purposes. The cellophane lasts about three months in a moist atmosphere, but it is safer to replace it about every ten weeks.

Plate I, fig. 1, shows the cages being used in a greenhouse in which feeding experiments and small cultures under lamp glasses are also being carried out.

## II. ARTIFICIAL FEEDING OF *MYZUS PERSICAE*.

It is of great importance in many problems of virus entomology to rear the insect vector apart from the host plant. This type of work has already been carried out by Severin and Swezy<sup>(2)</sup> and Swezy<sup>(4)</sup> for *Eutettix tenella*, using the methods of Carter<sup>(1)</sup>. The apparatus consists of a bag of "fish skin" (swim bladder) suspended in a cage and containing the medium which the *Eutettix* pierces the membrane to obtain. So far, however, it has not been used to culture aphides, which are also important as vectors of virus diseases. The Carter apparatus fails in that the aphides always crawl up to the roof of the cage and do not seem to recognise the damp globular surface as a substitute for a leaf. Obviously it is necessary to devise some means whereby the aphides may feed more or less in their natural position, which is upside down on the lower surface of a leaf.

Text-fig. 2 shows the first type of apparatus used. It is described in detail as, though limited in capacity, it is an easily prepared device and quite effective for certain types of work. It consists of a small crystallising dish (diameter  $1\frac{1}{2}$  inches) containing a little feeding fluid closed with a circle of washed fish skin held in place by an elastic band. A filter funnel (diameter 2 inches) is prepared for its reception by the construction of a small shelf of paraffin wax and the introduction of a few aphides,

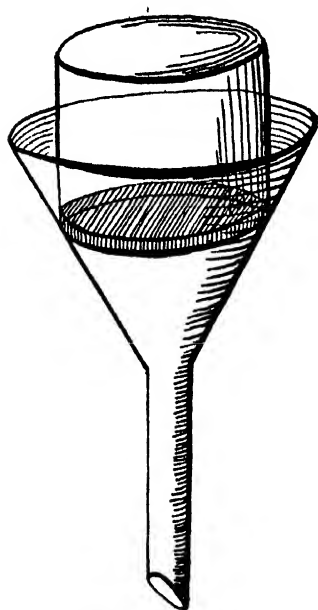


Fig. 2.

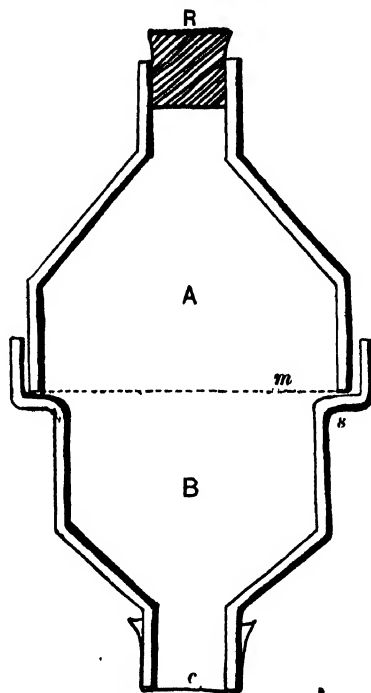


Fig. 3.

Text-fig. 2. Artificial feeding of *Myzus persicae*. Feeding apparatus, No. 1. Crystallising dish inverted over filter funnel.

Text-fig. 3. Artificial feeding of *Myzus persicae*. Feeding apparatus, No. 2. The crystallising dish and funnel replaced by specially constructed capsules.

the top of the stem being plugged with cotton-wool. The dish is inverted into the funnel and pressed down and the whole clamped in position. In this way aphides have been fed for six days on weak methylene blue and sugar solution and have produced young. This method, however, is not suitable for work on a large scale. Only a few aphides can be introduced as they cannot be kept in an open filter for more than a few seconds; also the apparatus cannot be kept in a glasshouse as it is very prone to "sweat" at high temperature. Only mixtures which are not likely to



decompose can be used because there is no way of replacing them, and extracted plant juices quickly break down and possibly become toxic to the insects. It is also advisable to use a very thin film, adding distilled water as it evaporates, pressure on the membrane causing it to leak. This cannot be done with a sealed capsule. On account of these difficulties the apparatus shown in Text-fig. 3 has been devised.

This apparatus consists of a pair of cone-shaped glass capsules *A* and *B*, their apices forming wide necks. *A* stands on a ground-in shelf (*s*) in *B*. It is covered with fish skin (*m*) as in the first apparatus (if fish skin is not obtainable gut skin can be used)<sup>1</sup> and contains the feeding fluid. By removing its rubber stopper (*R*) liquid can be changed or replenished without removing the capsule. Capsule *B* is perforated by four holes of 1 cm. diameter, some of which can be seen in the photographs (Plate XXXVI, fig. 2). They are covered with small circles of organdie muslin. In introducing the aphides the apparatus is inverted so that *B* is resting on *A*. The insects are placed on the membrane with a fine camel-hair brush through the neck of *B* which is then closed by a cap of muslin or cellophane (*c*). From 50 to 100 aphides can be introduced in this way without escapes. The apparatus is reversed and clamped into position as shown in the photograph (Plate XXXVI, fig. 2).

In replenishing the liquid the cage is inverted and the stopper removed. When the aphides have crawled into the upper part of *B*, the membrane can be rinsed with distilled water from a wash bottle. It is sometimes advisable to paint the edges of this membrane, when dry, with hot paraffin wax, as oozing takes place most frequently round the edge.

The mortality of *Myzus persicae* cultured in this way varies considerably with the strength and nature of the fluids and with the general conditions. Six days is the longest period for which they have remained alive (seven days in some cases for methylene blue), but it is hoped that the time will be much longer when the correct medium has been found. Using extracted potato juice the attempt to subculture on to seedlings is generally unsuccessful after the third day, though a few survive after the fourth. The subcultured aphides require a small glass cage with high humidity. That the insects actually feed and do not merely exist in the moist atmosphere is shown by the fact that stain can be detected in the gut after a few hours and many eventually become quite deeply coloured.

Table I shows the results of one set of cultures, but the numbers are

<sup>1</sup> Gut skin is a good deal thinner than fish skin and only the toughest pieces should be used. It can be obtained at Boots Cash Chemists, by special order.

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very variable, *e.g.* in methylene blue cultures the numbers remaining alive vary from 10 out of 12, to 7 out of 50, in six days. It represents fairly well the value of the different fluids as culture media.

Table I.

*Number of aphides alive on consecutive days.*

Medium	Days						
	1	2	3	4	5	6	7
Potato juice 25 % in distilled water ...	20	15	11*	8*	—	—	—
Methylene blue 0.05 % in sugar solution 0.05 %	20	17	15	12	8	5	3
Dahlia violet 0.01 % ...	20	18	16	16	13	10	—
Eosin 0.01 % ...	20	18	15	14	11	11	—
Light green 0.02 % ...	20	14	13	11	10	9	—
Eosin azure 0.01 % ...	20	13	7	3	0	—	—

\* Leaf-juice cultures were not carried beyond the third or fourth day as it was found impossible to subculture the two or three weakly specimens that survive till the fifth or sixth day. Eosin and methylene blue insects can be subcultured quite satisfactorily on the fifth or sixth day.

### SUMMARY.

1. A method for keeping pure and uninfected cultures of aphides for virus work is described; it involves the use of cellophane and bolting silk on a metal framework.

2. Specially constructed glass capsules are described in which aphides can be fed on artificial media, plant extracts or dyes.

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### EXPLANATION OF PLATE XXXVI

Fig. 1. Cellophane-covered cages on glasshouse staging

Fig. 2. Feeding apparatus, No. 2, in use.

(Received March 3rd, 1930.)



Fig. 1.

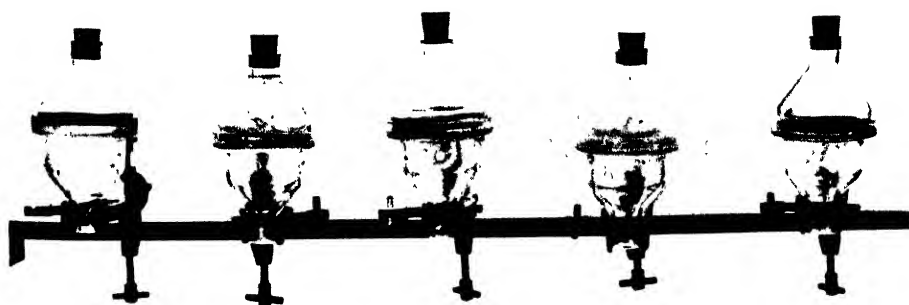


Fig. 2.



# THE RÔLE OF *THRIPS TABACI* LINDEMAN IN THE TRANSMISSION OF VIRUS DISEASES OF TOMATO

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## INTRODUCTION.

THE rôle of insects in the transmission of virus diseases of plants has long been recognised, but there is still uncertainty concerning the vectors of the virus diseases of tomato. White fly, *Trialeurodes vaporariorum* West, does not appear to transmit these diseases, Gardner and Kendrick (3) regard plant lice and flea beetles as possible carriers, while Olitsky (8) gives evidence of transmission of tomato mosaic by the "mealy" bug, *Pseudococcus citri*. Vanterpool (12) was able to demonstrate the transmission of tomato streak by aphides, but the latter writer does not give any details of the experiments or the insects used.

Many of the insects transmitting virus diseases belong to the order *Homoptera*, and possessing sucking mouth-parts to penetrate the plant tissues and feed on its juices, they would appear to be natural vectors of virus diseases. The possession of similar mouth-parts by members of the order *Thysanoptera*, and their prevalence both in the field and in glasshouses, has directed suspicion to these insects as possible vectors also. On account of their minute size and their habits they are difficult to control by insecticides and, therefore, are usually to be found in otherwise insect-proof glasshouses. In spite of these features they are not regarded as vectors by most workers and it was not until Pittman (9) obtained transmission of "spotted wilt" of tomatoes with *Thrips tabaci* Lindeman in Australia that serious attention was attracted to them. Schaffnit (10) mentioned *Thrips tabaci* Lindeman as a possible vector of mosaic diseases of beet and spinach, and Böning (1) regarded *Thrips flavus* Schrank as a possible vector of bean mosaic, but neither case has been confirmed.

The experiments described in this paper were carried out with *Thrips tabaci* Lindeman and virus diseases affecting tomatoes.

## MATERIAL AND METHODS.

(1) *Source of insects.*

These were obtained from a commercial glasshouse where they were found in large numbers on virus-free cucumber plants. Subsequently they were cultured on healthy tomato plants.

(2) *Source of plants.*

The tomato plants of Kondine Red variety used in these experiments were grown from seed under conditions as nearly insect free as is possible in an insect-proof glasshouse which was fumigated regularly. The plants were young and rapidly growing at the time when the infected thrips were placed on them.

(3) *Source of viruses.*

The viruses employed were from various sources, and each had been filtered through Pasteur-Chamberland L. 1 and L. 3 candles previously and artificially inoculated with a needle into successive series of tomato plants.

(i) *Tobacco mosaic* (= *Tobacco virus* 1 (7)). This came from Dr Grainger of Leeds University who obtained it from Dr Johnston of Wisconsin originally.

(ii) *Glasshouse streak*. The source of this inoculum was a commercial glasshouse. The plants showed the irregular, dark, necrotic lesions on the stems, petioles and leaves characteristic of the disease streak or stripe. In addition, the younger leaves of the plants showed the coarse mottle or mosaic usually associated with necrotic symptoms in streak. The virus producing this mosaic has been shown<sup>(6)</sup> to be indistinguishable from tobacco virus.

(iii) *Potato mosaic*. The virus of potato mosaic which produces regular, necrotic spotting on the leaves of tomatoes, as described by Henderson Smith<sup>(5)</sup>, was used in combination with (a) tobacco virus 1, and (b) the filtered extract of plants inoculated with glasshouse streak but showing the mosaic only. Both combinations produce a severe disease which is here termed experimental streak, in order to distinguish it from glasshouse streak, from which it differs mainly in the regularity and ease with which it can be transmitted by needle inoculation.

(4) *Methods.*

Young tomato plants growing in 6-inch pots were artificially inoculated with a needle about 10 days before each experiment, in order to provide a source of infection from which to infect the insects. A hurricane

lamp-glass chimney was placed over each plant, the base being firmly implanted in the soil, and the top covered securely with a piece of fine silk, thus forming an insect-proof chamber. Each plant was kept in its chamber while the thrips were colonised on it.

In each experiment, a certain number of insects were placed on infected plants and left to feed for varying periods of time, and then the insects were transferred to healthy plants in similar insect-proof chambers. This transfer had to be effected with great care, and to prevent any infected juice from broken hairs, etc. being carried over by the brush, with the insect, to the healthy plant. In order to eliminate this mechanical means of infection, the insects were taken off the infected plants with one camel-hair brush, placed in a petri dish, which was lined with black blotting paper so that the insects could be seen clearly and, with another clean brush, the desired number of infected insects were transferred to the healthy plants. After the insects had fed for the required time, the lamp glasses were taken off and the insects carefully removed from each plant, which was also sprayed with nicotine and soft soap.

In order to prove that the insects themselves were not carrying any disease from their source, suitable controls were set up with healthy tomato plants. In the second and third experiments, controls were also made to test the second series of brushes used in transferring the infected insects to the healthy plants. This was done by rubbing each brush over the young leaves of healthy tomato plants, different brushes having been used for each virus.

#### DETAILS OF EXPERIMENTS.

I. 7. v. 29. Fifty imagos and 50 nymphs were colonised on each infected plant for 7 days, and then varying numbers were transferred to healthy plants for a similar period.

Inoculum	No. of plants		No. of infected thrips on each healthy plant		Infection
	Infected	Healthy	Imagos	Nymphs	
Tobacco mosaic	2	2	(1) 16 (2) —	— 11	Nil
Glasshouse streak	2	2	(1) 17 (2) —	— 6	"
Glasshouse streak showing mosaic symptoms only	2	3	(1) 15 (2) 12 (3) —	— — 13	"
Experimental streak, No. 2	2	2	(1) 13 (2) —	— 4	"

II. 16. v. 29. Two hundred imagos were colonised on each infected plant for 6 days, and then varying numbers were transferred to healthy plants for a similar length of time.

Inoculum	No. of plants		No. of infected thrips on each healthy plant	Infection
	Infected	Healthy		
Tobacco mosaic	2	2	(1) 25 (2) 16	Nil
Glasshouse streak	2	2	(1) 25 (2) 15	"
Glasshouse streak showing mosaic symptoms only	2	2	(1) 25 (2) 19	"
Experimental streak, No. 1	3	2	(1) 25 (2) 12	"
Experimental streak, No. 2	4	3	(1) 25 (2) 15 (3) 9	"

III. 23. v. 29. Two hundred imagos were colonised on each infected plant for 5 days, and then a definite number were transferred to each healthy plant for 9 days.

Inoculum	No. of plants		No. of thrips on each healthy plant	Infection
	Infected	Healthy		
Tobacco mosaic	2	3	(1) 35 (2) 25 (3) 15	Nil
Glasshouse streak	2	3	(1) 35 (2) 25 (3) 15	"
Experimental streak, No. 1	4	3	(1) 35 (2) 25 (3) 15	"
Experimental streak, No. 2	4	3	(1) 35 (2) 25 (3) 15	"

Experimental streak, No. 1, was produced by combining the virus of potato mosaic in tomato, and tobacco virus 1.

Experimental streak, No. 2, was produced by combining the virus of potato mosaic in tomato, with the filtered extract of plants inoculated with glasshouse streak, but showing the mosaic only.

The number of healthy plants colonised with infected insects was low in each series, on account of the high mortality after the insects were transferred from cucumber to diseased tomato plants. The large area of necrotic tissue on the leaves of plants inoculated with forms of streak probably accounted for the death of the less active insects placed on these



plants. The mortality of the infected thrips after their transference to healthy tomatoes was low.

That the insects had fed readily on both the diseased and the healthy plants was shown by the number of white areas on the leaves where the epidermis had been punctured.

*Summary of insect controls.*

Control	No. of plants	No. of thrips per plant		No. of days on each plant	Infection
		Imagos	Nymphs		
Experiment I	5	50	50	14	Nil
Experiment II	5	100	—	12	"
Experiment III	5	200	—	14	"

In each of the above controls, the insects were colonised on young healthy plants showing three or four leaves, and enclosed under lamp glasses for a period of time equal to the duration of the corresponding experiment.

After the insects of each series in Exps. II and III were transferred from infected to healthy plants, the second brush used in the transfer was firmly rubbed over the young leaves of two healthy tomato plants. None of these plants developed any signs of disease, therefore we may conclude that no infection was carried on these brushes.

In the following table, the numbers of healthy plants used in the above experiments are grouped together according to the inoculum from which the infected insects colonised on them were derived.

Experiment	Tobacco mosaic	Glasshouse streak showing		Experimental streak	
		Streak and mosaic	Mosaic only	No. 1	No. 2
No. I	2	2	3	—	2
No. II	2	2	2	2	3
No. III	3	3	—	3	3
Total	7	7	5	5	8

Thus seven plants were colonised with thrips from plants inoculated with tobacco mosaic alone, and five from plants inoculated with tobacco mosaic combined with potato mosaic (= experimental streak, No. 1). In none of these cases was the virus transmitted, so that there are twelve cases of failure to transmit tobacco mosaic.

Seven plants were colonised with thrips from plants inoculated with glasshouse streak showing both typical necrosis and the mosaic, five with

thrips from plants inoculated with glasshouse streak but showing the mosaic only, and eight with thrips from plants inoculated with the latter combined with potato mosaic (= experimental streak, No. 2). Again there was no transmission, that is there were twenty cases of failure to transmit the virus of glasshouse streak.

If streak is a severe form of tobacco mosaic in tomatoes<sup>(6)</sup>, then there are, in all, 32 (12 and 20) cases of failure to transmit tobacco virus 1.

Five plants were colonised with thrips from plants inoculated with potato mosaic combined with tobacco mosaic (= experimental streak, No. 1), and eight with thrips from plants inoculated with potato mosaic combined with the extract of plants inoculated with glasshouse streak but showing the mosaic only (= experimental streak, No. 2). As there was no case of transmission, there are 13 failures to transmit potato mosaic in tomatoes.

#### DISCUSSION.

The failure of the above experiments to demonstrate transmission of these viruses is somewhat surprising, when one considers that Pittman readily obtained transmission of "spotted wilt" of tomatoes in Australia, using only three to five nymphs per plant.

It is, of course, possible that the Australian disease, "Spotted Wilt," is not identically the same disease as English streak, for neither Brittlebank<sup>(2)</sup>, Hamblin<sup>(4)</sup> nor Pittman<sup>(9)</sup> has succeeded in transmitting "spotted wilt" by artificial inoculation of extracts from diseased into healthy plants. On the other hand, Brittlebank<sup>(2)</sup> concludes that "spotted wilt" in Australia is probably identical with winter blight of tomatoes in America, as described by Selby<sup>(11)</sup>, and Vanterpool also regards these diseases as identical. The American disease corresponds in all particulars to the streak or stripe found in England. From personal observation of "spotted wilt" in Australia, the writer regards the disease as indistinguishable from the English disease. The comparison of these diseases is made purely on symptoms, and it is recognised that symptoms alone are not a safe basis for identification, but as yet no other criteria are available in the case of "spotted wilt."

Again, it is possible that the insect, *Thrips tabaci* Lindeman, used by Pittman was of different strain and habits to the English insect of the same identification used in these experiments. Moreover, the conditions such as light, temperature and humidity under which these experiments were conducted may not have been favourable to transmission of the disease by the thrips, although they feed freely on the plants. It is clear

that *Thrips tabaci* does not transmit virus diseases of tomato under all conditions.

However, one positive result such as that of Pittman is of more value than many negative ones, and if it cannot be repeated, either the conditions or the materials with which it was obtained have not been completely reproduced, or it is due to unnoticed factors which can only be detected in repetition of experiments.

#### SUMMARY.

A description is given of experiments designed to show the rôle of *Thrips tabaci* Lindeman in the transmission of tomatoes.

The diseases tested were tobacco mosaic and glasshouse streak singly, and the viruses of each of these two combined with a potato mosaic virus to give a disease termed experimental streak.

The source of the materials used and the methods employed are described in detail.

In no case was transmission of any of the viruses recorded, although the insects had fed freely on all the plants. It is concluded that *Thrips tabaci* does not transmit virus diseases of tomatoes under all conditions. The importance of this insect as a vector of these diseases in commercial glasshouses in England is therefore doubtful.

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# THE PHYSIOLOGY OF VIRUS DISEASES IN PLANTS

## I. THE MOVEMENT OF MOSAIC IN THE TOMATO PLANT

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(With Plate XXXIII.)

### INTRODUCTION.

THE study of virus diseases has, in the last few years, become of great importance. It has been increasingly recognised that many of the common diseases both of plants and of animals are attributable to agents called filter-passing viruses. At present, the main criteria of a virus disease are the symptom-complex induced, its infectivity and the invisibility of the causative agent. The structure of the causative agent is, in the nature of things, not at present recognisable. The general position of virus diseases has been discussed recently by various authors, and reference may be made to their papers for details.

Recently attempts have been made to assess the physical characters (resistance to heat, to alcohol, etc.) of the infective principle and a beginning has been made with the study of the physiological aspects.

As virus diseases are recognisable mainly by their symptoms and their infectivity, these are the two aspects of the problem which have been most studied under laboratory conditions. It is clear, however, that symptoms do not serve as very reliable indices of this type of disease on account of the inherent difficulty of rigidly defining a disease by symptoms which may, under differing environmental conditions, vary between wide limits.

As this uncertainty constitutes a real difficulty, it was decided to attempt, in this laboratory, some more general studies on the physiology of virus diseased plants. Various lines have been opened up, and the one with which this paper deals is that of the movement of the causative agent within the plant. This is an aspect which has been little studied. It was felt, however, to be of considerable importance in that it may be possible to give some indication of the nature of the causative agent if it be definitely shown how its movement through the plant takes place.

## RESULTS OF PREVIOUS WORK ON THE MOVEMENT OF VIRUS.

The few data relating to this problem are rather scattered through the literature. Bennett<sup>(2)</sup> was one of the first workers to examine the actual tissues through which movement takes place. He investigated the movement of the curl virus of raspberries through "ringed" stems. From his experiments he concluded that (1) the curl virus can be confined to the inoculated shoot for an indefinite period by the simple process of "ringing," (2) a relatively small amount of "bark" bridging a "ring" is sufficient to permit of the passage of the curl virus from the inoculated part to the parts below the "ring." He suggested that the movement of the virus from the root of the resting stool "may parallel the movement of food."

Some time earlier Severin<sup>(14)</sup> had attempted to determine the rate of movement of the infective agent. He found that the virus of curly-top of sugar beet moved in "one-half hour at a mean temperature of 103.5° F. through the petiole 7 inches long." This was the quickest rate he obtained. More usually the rate was of the order of 4 inches per hour in his experiments. It was also found that the disease was transmitted from infected adults of *Eutettix tenella* feeding on the outer leaves of a plant to non-infected individuals on the inner leaves of the plant in 6 days. When the positions of the leaf-hoppers were reversed, the movement outwards took 10 days, at a slightly lower temperature.

The infective agent of curly-top was transmitted from infected hoppers on one of the first two leaves of a young beet plant to non-infected males on the opposite outer leaf at the end of 2 days at 81° F.

McCubbin and Smith<sup>(11)</sup> inoculated the main stem of tomato plants, the lower branches of which had been "layered" so as to induce rooting. By separating the daughter-plants after various intervals of time, they found that the virus agent travelled from 8 to 18 inches in 10-20 days. The virus agent apparently left the main plant between the 3rd and the 10th days.

Priode<sup>(12)</sup> found that, in the ringspot disease of tobacco, lesions frequently appear in the region of the inoculated leaf. Usually systemic infection follows. Holmes, in two papers<sup>(8, 9)</sup>, has shown that the virus of tobacco mosaic may be found subsequent to inoculation above the inoculated leaf before it appears below. He has found that inoculation through broken trichomes takes place instantaneously, and that immediate washing off of the infected juice is not sufficient to prevent infection. Rubbing the infected juice on to wounds made some time before did not result in the appearance of symptoms.

Storey(17) found that the movement of the virus of the mosaic disease of maize across or down the leaf was not obviously impeded by cutting out portions of the midrib or by severing the veins of the half-lamina in which inoculation took place. "This result," he writes, "was to have been expected, for in either case the leaf remained turgid, being supplied by the small anastomosing veins which form a network through the leaf lamina." He found that movement down a leaf, subsequent to insect infection, was at a rate varying in six cases out of sixteen from 10 cm. to 20 cm. per hour.

Böning(4) found that the virus of mosaic disease travelled in tobacco leaf a distance of 13 cm. in a minimal period of 2 days and in tomato leaf a distance of 9 cm. in 2 days. In the tomato leaf "streak" travelled a shorter distance in 4 days. His results for rate of movement up and down the stem may be summarised in the following way. The results refer to tomato stems.

Table I.

*Movement of virus agents in tomato stem (from Böning).*

<i>Mosaic.</i>			<i>Streak.</i>		
Direction	Distance (cm.)	Time (days)	Direction	Distance (cm.)	Time (days)
Down stem	12	4	Down stem	12	6-7
Down stem	20	4-5	Down stem	20	4-5
Up stem	12	3-4	Up stem	12	4-5
Up stem	25	4-5	Up stem	25	5

It will be seen that there is some variation in the results, but they all do definitely suggest that the movement of the virus is comparatively slow, and that it moves in either direction in the plant at rates of the same order of magnitude.

Recently, Davis(7) has summarised the literature on the Infectious Chlorosis of Variegated Plants. He deals especially with the work of Baur and of Lindemuth. Baur found that the causative agent of chlorosis in *Abutilon avicennia* apparently moves through the extra cambial tissues. He prevented the movement by "ringing" the stem. He also suggested that the agent could move through an immune *A. arboreum* stem grafted on an *A. avicennia* without multiplication—the former stem being a mechanical means of transport. On the other hand, Blakeslee(3) has shown that the agent of the "Q" disease of *Datura* did not travel through a *Petunia* stock on an infected scion.

## THE PROBLEM HERE TREATED.

The evidence adduced from the experiments on the rate of movement gives one but little indication of the tissues in which the movement is taking place. It was proposed, therefore, to deal with that aspect of the problem.

Various general considerations operated in suggesting the lines along which work might be carried out. It has already been shown by various authors (Auchter(1), Curtis(6), Caldwell(5)) that, when substances moved in the phloem or in the xylem either upwards or downwards in the plant, movement was considerably more free in a vertical than in a lateral direction. The movement laterally was much less rapid, if, indeed, in some cases it took place at all.

The first experiments were, therefore, set up with a view to studying localisation of the movement of the virus in the tissues of the plant. When an eosin solution was absorbed by a petiole stump, subsequent to the removal of the lamina, the eosin was found to travel upwards and downwards on the same side, and to pass over to the other side of the plant only after it had travelled out to and round the anastomosing vessels at the leaf-tips. In plants with decussate phyllotaxis the localisation was particularly well marked (see Caldwell(5)). A simple analogy suggested itself with the substitution of virus inoculum for the eosin solution. All the experiments hereafter described were carried out using the "aucuba" disease of tomatoes—a mosaic disease which has been described in detail by Henderson Smith(15). This disease was chosen for the work in this investigation because it is easily transmitted by juice inoculation, is very infectious, and shows particularly well-defined symptoms. The symptom-complex has already been discussed by Henderson Smith, who described the main features, in bright weather under normal conditions, as follows: "Scattered over the leaf are patches of white and patches of yellow, usually sharply delineated but sometimes shading into neighbouring areas, irregular in shape and size, often angular, and occurring in all parts of the leaf." This disease is so infectious that it is easily transmitted merely by rubbing a healthy plant after infected plants have been handled. This feature is especially valuable when dealing with experiments yielding negative results, as, in the main, symptoms regularly follow inoculation.

Inoculations were made as follows: leaves from a plant showing aucuba symptoms were cut into small pieces and crushed in a mortar. Thereafter was added a volume of distilled water equivalent to twice the



weight of the leaf material. The whole was carefully pulped and mixed. A few drops of this material were transferred by means of a Pasteur pipette to the back of a marking label. This was held under the pinna to be inoculated, and another drop was placed on the upper surface. This drop was carefully spread over the whole of the upper side of the leaf. Some 30–40 holes were then pricked in the lamina—care being taken to distribute them as evenly as possible. In all cases where the plant as a whole was to be infected, four pinnae were treated, but in some cases—as when unilateral infection was attempted, only two pinnae (both on the same leaf) were so treated. All the apparatus used was sterilised so far as was possible, and the whole operation was carried out with the minimal chances of accidental infection.

#### INOCULATION ON ONE SIDE.

It was thought from analogy with the movement of metabolites that, if the movement of the virus agent was localised in the vascular elements, the tendency would be for symptoms to appear in the region of the treated shoot or leaf. Especially should the leaves on the same orthostichy show symptoms rapidly. As has before been pointed out, the axillary shoot has similar vascular connections to the leaf in the axil of which it develops. In the main, symptoms appear first on the younger leaves which are, naturally, at the top of the plant. As the axillary buds develop, however, their leaves also show symptoms.

In the first experimental plant  $C_2$  inoculation was made on July 8th on the leaves of a single branch. Symptoms appeared at the top of the plant and on the leaves of the axillary bud above the treated shoot on July 21st. Two other shoots on the same side showed symptoms by July 22nd. The symptoms appeared on the leaves of the opposite axillary shoots on July 26th. There was in this case, therefore, some delay in the appearance of the symptoms on the side away from the inoculation.

In the other plants,  $C_1$ ,  $C_3$ , inoculated at the same time and on leaves of a single branch, the symptoms appeared first at the top and then downwards as the axillary bud-leaves grew without any apparent localisation. Similar results were obtained with a fourth plant,  $C_9$ , which was inoculated on July 18th. Four plants,  $A_5$ – $A_8$ , inoculated on July 18th and on a single leaf all showed the symptoms systemically. Among four other plants,  $R_9$ – $R_{12}$ , inoculated similarly on July 24th, three showed systemic infection, and in one there was a slight delay on the side away from the inoculation. Four large plants similarly treated by Dr Henderson Smith in October showed symptoms quite systemically. Out of

sixteen plants, therefore, one showed a delay of 4 days in the appearance of the symptoms on the further side as compared with the nearer, and one case had a doubtful delay of 1–2 days. The others were characterised by systemic infection. When it is borne in mind that the time elapsing before the appearance of symptoms is at all times rather variable, and may vary from 4–18 days under not dissimilar circumstances, the slight delay found in these cases may hardly be considered as significant.

Having been unable to demonstrate definitely the localisation of the movement of the virus to the side on which inoculation was made, another type of experiment was set up.

#### EFFECT OF "RINGING" THE STEM.

The results obtained by Baur (Davis(7)) and by Bennett(2) suggested that the effect of "ringing" was to stop the movement of the infective agent. This did not appear to be so in the case of tomato, as in all of the four tomato plants tried symptoms did appear on the parts of the plants on the non-inoculated side of the "ring." In this plant regeneration takes place very quickly—the stelar tissue is essentially parenchymatous in nature. (There is further a feebly developed intraxylary phloem in the tomato.) As a consequence of this it was difficult to decide whether the symptoms appeared as a result of infection by a virus which had moved very slowly through the living cells in the xylem and the pith, or else had travelled through the phloem tissue which arose as a result of the regeneration of the tissues.

#### EXPERIMENTS WITH PARTIALLY KILLED STEMS.

As it was not possible to rely on the non-recovery of the "ringed" stem, an attempt was made to remove the living cells in the stem by killing them. The agent used was chloroform, which destroys the protoplasm of the parenchymatous cells and which has, apparently, but little effect on the virus of aucuba mosaic (see Henderson Smith(15)).

A region in an internode about the middle of a fairly large stem some 40 cm. high was killed over a distance of 5 cm. This was done by making two incisions at right angles through the stem with a sharp scalpel. The incisions were 1–2 cm. long. Into them was put chloroform which was also applied to the outside of the stem. The plant was first staked up and lightly tied to the stake. After some hours the outside of the treated portion of the stem was smeared with vaseline. The effect of the treatment with chloroform was seen rapidly. The tissues almost immediately lost their turgidity and at first looked as if they were waterlogged. By the

next day the treated area and a portion on either side of it had become brown and quite dead. On that day the plants were inoculated either above or below the lesion. In other cases the stem treated was that of a branch, and the distal end of the branch was treated as being "above" the lesion.

In these plants which had been so treated, the effect of the killing of the living tissue of the stem early became apparent. Adventitious roots appeared above the lesion, especially on the region immediately above the killed tissues. The appearance of one such plant is illustrated in Plate XXXIII, fig. 1. The results are given below in tabular form. Four experiments were carried out with branches treated and twenty-two with stems. In some cases the inoculation was made "above" the lesion, and in others "below." The plants were all inoculated between July 1st and July 24th, 1929.

Table II.

*Details of plants inoculated after the stem had been treated with chloroform.*

Total number of treated plants (July 1st-24th, 1929)	26
No. of plants when infection did not pass lesion	14
No. of plants when infection was not delayed by lesion	4
No. of plants where tissues partially recovered	4
No. of plants where symptoms appeared across lesion	4

It can be seen in this table that fourteen plants showed no symptoms on the opposite side of the lesion. In twelve plants symptoms did appear across the lesion. There is good evidence to account for this result in the case of eight of the plants. In the four plants where "the infection was not delayed by lesion" the symptoms appeared simultaneously on either side of the lesion. It was, therefore, presumed that the "killing" of the tissues had not been quite complete and that some living cells had remained. In the four plants of the next section there was regeneration of the tissues secondarily, so that long after the symptoms had appeared on one side of the "killed" tissue the infective agent was able to move across the regenerated tissue, and to cause the appearance of symptoms on the opposite side. In the last four plants the symptoms appeared after a delay of 4-5 days and the tissue had not regenerated. It is suggested that these plants were probably accidentally infected by handling.

The whole experiment was repeated on August 23rd and 28th and September 4th-5th, 1929. The plants in this case were not now treated with chloroform, but the stems were treated with steam in a fashion similar to the chloroform treatment. A piece of damp cotton-wool was

wrapped round the stem at the middle of a suitable internode. This cotton-wool in turn was held in place by a small sheet of tin-foil wrapped round it. The outside of the tin-foil was heated with a small gas jet and the water on the cotton-wool boiled. Care was taken to protect the leaves above and below the treated internode. After this treatment the plants were left for one day, after which the outside of the dead area was vaselined. The stems in these plants again were tied to canes to prevent their falling. Inoculation was made below the killed tissues in each case.

In the first three plants, *S* 21–*S* 23, symptoms appeared below the lesions in 5 days. In *S* 24–*S* 26 they appeared in 8 days. In the plants *S* 30–*S* 44 the symptoms appeared between the 5th and the 7th day after inoculation. Untreated controls showed symptoms on the 5th day. Only one out of these plants showed symptoms above the killed area.

Table III.

*Data relating to the plants with "steamed" stem.*

Total number of plants	21
No. of plants showing no symptom above lesion	20
No. of plants showing symptoms above lesion	1

The plants which were used for the experiments had, unfortunately, to be tested for latent virus by being kept for 4 weeks or so. It was not possible to keep the portions above the lesions turgid for as long a time, and usually the leaves tended to wilt about the 10th day after treatment. For this reason, it was necessary to remove the upper portion of the plant just above the killed area at the end of a fortnight and to grow it separately as a cutting. The cuttings were then examined daily for symptoms.

That the xylem water current had not been cut off by the treatment received was indicated by one or two observations. (*a*) The upper portion of the treated plants remained quite turgid for over a week, usually for a fortnight. (*b*) The xylem vessels appeared quite open on sectioning the tissue. (*c*) The shoots elongated after treatment and appeared quite normal. In the case of one of the treated branches measurements were made on two of the leaves distal to the killed tissue. The figures are given below:

Table IV.

Leaf	Length on July 3rd (mm.)	Length on July 10th (mm.)
<i>A</i>	22	37
<i>B</i>	35	60

It will be seen that in one week the increase was nearly 100 per cent. Another and probably more satisfactory method of determining if water were passing up the stem across the dead area was the following. The stem of a treated plant was removed just above the roots and the cut end immersed in a solution of eosin, or some other dye. The solution travelled up the stem and passed across the treated area without obvious delay. Similar results were obtained when the dye was absorbed at the cut end of a petiole either above or below the lesion. The solution passed from below upwards or from above downwards quite readily—the dead tissues offering no apparent obstacle to movement. The movement of Chinese ink has taken place up to 3 weeks after the killing of the tissues.

#### MOVEMENT OF PARTICULATE SUBSTANCES THROUGH KILLED STEMS.

Of special interest in this connection are the experiments with diluted Chinese ink and nigrosin in water. When the cut stem was placed in either of these substances, the movement through the killed area of the stem could readily be watched by noting the blackening of the vascular bundles. The movement of these materials was not stopped at the top of the lesion but, on sectioning, particles of them were found in the vascular elements of the distal portion. This proves that the vessels were not plugged with protein materials, which might have acted as colloidal filters preventing the free movement of a virus body while allowing the possibly smaller eosin molecules to pass across.

Snow (16) has found that very similar treatment in the case of bean seedlings killed the living tissues but did not, at first, impede the movement of water. He found that the distal portion of plants in which an area of stem had been killed remained turgid for some 3 weeks.

The upper portion of the plant rooted readily as a cutting, and showed symptoms comparatively rapidly after being established if it had been previously infected. Those from treated stems were grown as plants for 4 weeks before being considered as clean. The cutting was always taken just above the dead tissue.

To ensure that the time (14 days) which elapsed between inoculation and wilting was sufficient to admit of the movement of virus up a normal stem, various untreated plants were inoculated. In some of these cases the minimal dose was given—inoculations being made on a single pinna at the base of the plant. After 4, 5 or 6 days the tops were removed some five or six internodes above the inoculation. Only one of the 4-day plants did not show symptoms on the upper portion which had been treated as a cutting.

Further, controls of the same size as the treated plants, inoculated at the same time but without steamed tissue, were arranged. In every case symptoms appeared, at the top of the controls, before the distal portion of the treated plants had wilted.

The mere fact of using the tops as cuttings to test if the virus were present in them is not in itself sufficient to inhibit the development of symptoms in infected material. It has been pointed out that infected stems will root and show symptoms comparatively rapidly. If young leaves are allowed to root on sand, it has been found by the writer that subsequent inoculation on one pinna results in the appearance of virus symptoms over the whole leaf. Purdy<sup>(13)</sup> found similar results with tobacco leaves. To ensure, further, that no top was a "carrier," the leaves of some of the tops were removed at different stages and inoculated, as above described, into batches of four young actively growing plants. In no instance did the test plants develop symptoms. Incidentally, no case of a "carrier" for aucuba mosaic has yet appeared in our cultures.

To determine if any virus had passed into the dead tissue and had there been adsorbed, the following inoculations were made. The region of dead tissue was divided into three so that the middle portion was quite free from the living tissue above or below. The three portions were approximately equal. Inoculations of each of these three portions were made into sets of plants. In the first case, the lower third, all the plants developed symptoms, but in the other two sets all the plants were clean.

#### POSSIBLE ADSORPTION OF THE VIRUS AGENT.

One possibility suggested itself in the matter of the non-crossing of the killed area. It was thought that the actual killing of the tissues might have liberated some substance which inhibited the development of symptoms above the lesion, or, alternatively, the dead tissue might have absorbed the virus agent and prevented its further passage. To ascertain if either of these suggestions were probable, the following set of experiments was carried out.

A quantity of macerated leaf-tissue from virus plants was divided into three portions. One portion was inoculated directly into the first set of plants. These served as controls. The second portion was centrifuged at a high speed for 5 minutes; then thoroughly shaken up; again centrifuged for 10 minutes and thoroughly shaken. After having been centrifuged for another 10-minute period the supernatant liquid was decanted. The residue was mixed with a little sterilised distilled water. The super-

natant liquid was a clear brown colour. Sets of plants were inoculated with the supernatant liquid and with the residual material.

The third portion of the infective material was passed through muslin to remove the larger pieces of tissue. To the filtrate was added a quantity of stem tissue of uninfected plants which had been steamed in the manner previously described. The whole was carefully mixed together. The mixture was treated in exactly the same way as was the green tissue. It was alternately mixed and centrifuged. Thereafter the supernatant and the residual material were inoculated separately into sets of plants. There were, therefore, five sets of plants, viz.

- (a) Controls inoculated with untreated macerated material.
- (b) Plants inoculated with supernatant liquid of centrifuged macerated material.
- (c) Plants inoculated with residue of centrifuged macerated material.
- (d) Plants inoculated with supernatant liquid of centrifuged mixture of infected juice and boiled tissue.
- (e) Plants inoculated with residue of centrifuged mixture of infected juice and boiled tissue.

These plants were set up on October 16th, 1929. Most of them showed symptoms of aucuba mosaic by October 29th. All were definitely infected when they were discarded on November 29th. The effect of the boiled tissue, therefore, was not appreciable under the conditions obtaining in these experiments.

#### DISCUSSION.

From the results of the experiments detailed above, it is evident that one point has been established regarding the movement of the virus of aucuba mosaic in tomato. It has been clearly demonstrated that the virus agent did not travel across tissue of which the living elements have been killed. Through this tissue, on the other hand, water could and did pass. The xylem vessels were not blocked, and coloured solutions passed freely upwards or downwards. No adsorption or inhibition of the causative agent could be demonstrated. One is, therefore, forced to the conclusion that the virus agent cannot travel mechanically in the xylem stream, but can only pass through the living tissue. For some reason, as yet not understood, survival of the tissue is necessary for the movement of the virus (cf. Holmes(9)). This is of more than passing interest, when it is remembered that the virus of tobacco, for example, can withstand the curing processes. In my own experiments, I have found that the

dead, dry leaves of aucuba infected plants continued to be infective when inoculated into fresh plants.

The conclusion is in complete agreement with that of Baur and of Bennett. It does not confirm the implied suggestion of Storey, who pointed out that it was not surprising that the virus travelled across a cut lamina, since the opposite side was still turgid. The implication is that, if the water passed across the leaf, the virus should also have passed. This is not necessarily so. Auchter has demonstrated, and I have confirmed his results, that there is distinct localisation in the movement of salts, etc., in the plant. It appears that, in the main, rapid lateral movement in plant tissues is confined almost entirely to water; even water-soluble salts do not appear to travel so rapidly.

It may, therefore, be stated definitely that, for the virus principles which have been studied, there is no direct evidence for movement in the xylem. All the experiments have, so far, shown that movement does not take place through the xylem tissues.

#### MOVEMENT IN THE PHLOEM.

Movement through the phloem is more difficult of demonstration. The evidence adduced on this point must, in the nature of things, be circumstantial. It is not practicable to isolate "phloem" from "living" tissues. Two lines of argument may be pursued. If the movement were necessarily confined to the phloem tissues, there would be, it is suggested, some evidence of localisation of the symptoms to one particular portion of the plant. The leaves on the axillary shoot of the inoculated leaf and those directly above and below would, presumably, show symptoms first. The leaves on the alternate sides, that is, those with petioles more or less at right angles to the treated leaf, would next develop symptoms. The basis of this argument and the data concerned are contained in the papers on the movement of materials in plants (Caldwell(5)). The leaves on the side opposite to the treated leaf should not develop symptoms until long after the others. If movement were extremely rapid this might not necessarily hold. It has been shown, however, that it is not.

In the experiments recorded above, only one somewhat doubtful case occurred in which symptoms appeared some few days later on the opposite side. Even if this were a valid instance it is isolated, and, in any case, the difference in time is much too small to have any great value. The general conclusion to be derived from the experiments here recorded and from numerous others is that in the tomato, at least, there is no evidence whatsoever for localisation of movement to any sector of the plant. Severin



actually reports cases where the virus had, apparently, passed down the petiole of a leaf, had crossed the developing "bulb" of a beet, and passed up the petiole of the opposite leaf. The whole operation, including, presumably, some multiplication in the tissues of the opposite leaf, had occupied 2 days. This suggests, in the light of the work referred to above, that movement must have taken place across tissues other than phloem.

The rates at which movement takes place in the tissues are of value in this connection. Storey and Severin have measured movement within a petiole or within a leaf-blade. The data they report are given in the following table.

Table V.

*Rate of movement of the virus of sugar beet and of maize.*

Time (hr.)	Virus	Plant	Distance travelled (cm.)	Author
$\frac{1}{2}$	Curly-top	Beet	17.5	Severin
$\frac{1}{2}$	"	"	8.1	"
1	"	"	8.75 (twice)	"
1	"	"	11.8	"
1	"	"	13.1	"
1	Mosaic	Maize	10 (thrice)	Storey
2	"	"	40 "	"

In Table I Böning's data for tomato mosaic and for "streak" have already been given. In my experiments rates of the same order of magnitude were obtained, though there is always some variation due to individual differences in the plants used. There is no reason to assume from these data that the agent was moving in the xylem: rather there is strong evidence that it was not. It is difficult to believe that the infective principle could move so slowly in the water stream. Most experiments suggest a rate of some 2 mm. per hour, following needle inoculation. Any data available suggest a very much greater rate of movement for water in the xylem tissues (cf. Sachs, etc.).

Movement in the leaf tissues appears to be very much faster. In this case the material used was inoculated into the tissues by insect-vectors. These insects are known to penetrate by means of their stylets right to the phloem tissues. It is, therefore, suggested that the few observations which give very quick rates of movement were made when fortuitously the infective insect had inoculated its virus right into the phloem of one of the larger veins and when the second insect had sucked the infective juice out of the same vein. In the majority of cases, however, movement takes place from cell to cell by some mechanism of diffusion. In cases

where the phloem is injected and not seriously upset movement is more rapid (cf. Mason and Maskell<sup>(10)</sup>). This probably accounts for the isolated cases which periodically occur in work with the tomato, where symptoms appear within 4 days of inoculation. As has before been noted the symptoms first appear in the rapidly developing apical leaves.

The following general conclusion must, therefore, be drawn as a result of a consideration of the work above, viz. that there is no evidence of movement in the xylem. Direct evidence indicates that movement does not occur there in the case of, at least, three types of virus agent. Bennett has found that the curl virus of raspberry will not travel across ringed stems. Baur obtained similar results with the infectious chlorosis of *Abutilon*. I have, in this paper, shown that the causative agent of *aucuba* mosaic of tomato does not cross regions of stem where the living tissues have been killed.

As for the extra-cambial tissues, the absence of any apparent difficulty of lateral movement of the virus agent in the plants points to there being no inability on the part of the virus to travel in any living tissue. Direct evidence from inoculation through trichomes supports this view. On the other hand movement may, on occasion, be so rapid as to indicate that it must have taken place in the phloem elements.

#### SUMMARY.

In this paper the movement in the plant of the causative agent of virus disease is discussed. The relevant data in the literature are summarised.

A method is described whereby a portion of the stem in the middle of a tomato plant was killed either by chloroform or by steam. In this way the living upper and lower portions of the plant were connected by a bridge of dead tissue. It is shown that the symptoms appeared in that part of the plant in which the inoculation was made. The virus agent did not travel across the dead region.

The xylem tracts were not materially affected by this treatment, and water travelled across the region. Evidence of this is the fact that the distal portion remained turgid and sometimes continued growth for a considerable time. If the stem were removed above the ground level and put into eosin solution, this travelled readily over the dead tissue. That the vessels were not occluded by protein plugs is shown by the fact that particulate substances were carried up the xylem tracts past the dead region.

No evidence of adsorption of the virus agent to the cell remains could be adduced, so it is assumed that it was not travelling in the xylem stream.



CALDWELL.—THE PHYSIOLOGY OF VIRUS DISEASES IN PLANTS (pp. 429-443).



From previous experiments, it is known that the movement of metabolites and of stains tends to be localised to the side of the plant into which they are introduced. It was found that inoculation with juice of diseased plants caused systemic infection in all the treated plants. There was no apparent localisation of movement such as would have been expected had it been taking place through the vascular system. From this, and from other evidence in the literature, it is concluded that movement takes place in the living ground tissue of the plant.

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## EXPLANATION OF PLATE XXXIII

In this plant the stem at one internode had been "steamed." Subsequent to inoculation below symptoms appeared on the lower part of the plant while the upper part remained healthy.

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# THE PHYSIOLOGY OF VIRUS DISEASES IN PLANTS

## II. FURTHER STUDIES ON THE MOVEMENT OF MOSAIC IN THE TOMATO PLANT<sup>1</sup>

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(With Plates XVII-XX and 1 Text-figure.)

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### INTRODUCTION.

IN the first paper of this series, the results of some experiments on the movement of the agent of the virus disease—*aucuba mosaic* of tomato—in tomato plants were described and discussed. It was shown that the agent tended to move freely through the plant, that the movement was apparently not restricted to the vascular tissue and that no movement took place through dead tissue or, normally, in the water stream in the xylem vessels. When a portion of the stem was steamed so as to destroy all the living tissues in the treated area the virus was found to be unable to cross that region and to infect the upper part of the plant if it had been inoculated into the lower, and conversely. When Chinese ink was introduced into the xylem vessels in the lower part of the stem, particles were

<sup>1</sup> A grant in aid of publication has been received for this paper.

found to pass into the normal upper part of the plant, across the steamed area. This demonstrated that protein plugs had not been laid down in the vessels; such plugs might conceivably have filtered the virus out of the upgoing water stream. The data obtained suggested that the virus agent of aucuba disease of tomato did not travel, as do the normal metabolites, chiefly in the vascular tissues proper, that movement could take place freely through any living tissue, there being no localisation of the movement as was found in the case of dyes, metabolites, etc., and that movement through non-living elements was normally not possible. This line of investigation has been continued, and that paper deals with the results of these subsequent groups of experiments.

#### EXPERIMENTS WITH TOBACCO MOSAIC IN TOBACCO AND IN TOMATO.

The experiments with aucuba disease of tomato were repeated with tobacco mosaic in tobacco and in tomato. The variety of tobacco used was *N. tabacum* var. White Burley, that of tomato *S. lycopersicum* var. Kondine Red. The aucuba mosaic material was the standard material of this station, originally obtained from Dr Bewley at Cheshunt. The tobacco mosaic had been obtained indirectly from Dr Johnson and was his No. 1 strain. The same procedure was adopted in these experiments as in the previous ones. An internode of a tomato stem was steamed in the manner described in the earlier paper, so that all the living cells were killed without interfering to any great extent with the water stream in the xylem vessels. Thereafter, inoculations were made from macerated tissue of leaves from a tomato plant infected with tobacco mosaic. In this case, as before, when the inoculation was made in the lower part of the plant, the symptoms appeared in that part: when the inoculation had been made above, only the upper part of the plant was affected. There were no cases in which infection had spread across the lesion. Even when the upper uninfected part of a plant inoculated below the lesion was removed, planted as a cutting, and allowed to grow for some weeks, on no occasion did symptoms appear in that portion. The top of the plant was removed above the lesion by cutting with a red-hot scalpel. This had the effect of searing the cut end and precluded the possibility of accidental infection of the living cells. The tomato cuttings "strike" very easily since the root initials are well formed before the stem is cut. The appearance of a stem with a cluster of roots above the steamed portion is shown in Plate XIX, fig. 3.

In the experiments with tobacco plants, even more care was required than in those with tomatoes. The internodes of tobacco plants at the



stage most suitable for these experiments are rather short. Further, there is no great development of mechanical tissue in the stem of this plant. As a result of these two factors, when the stems had been steamed, the leaves of the upper portion tended to fall over on to the lower. In tomatoes, on the other hand, the stems of plants  $1\frac{1}{2}$ –2 feet high are sufficiently well provided with mechanical tissue to permit of their remaining rigid after treatment with only slight support. It is the more important to prevent the upper and lower leaves of tobacco from rubbing together in these experiments, since the thick mat of glandular hairs which invests the upper and lower sides tends to make the separation of two leaves lying together a rather delicate task if the hairs must not be broken. It should be borne in mind that aucuba mosaic of tomato and simple tobacco mosaic, both in tobacco and in tomato, are so contagious that damage to the trichomes under non-sterile conditions may lead to the infection of the plant. To prevent any difficulty arising from these considerations the upper part of the plants were enclosed in paper collars to prevent the drooping of the upper leaves on to the lower. When this was done it was found that the upper leaves remained fresh for a period of a fortnight and that, thereafter, the upper portion of the plant might be planted as a cutting. With these plants, again, it was found that no movement of the virus agent took place across the lesion. This fact brings this virus, which is closely similar in other respects to that of aucuba mosaic, into alignment with it as regards the methods of translocation in the plant.

#### EXPERIMENTS ON THE ABSORPTION OF VIRUS JUICE THROUGH A CUT PETIOLE.

It has been shown that solutions may be sucked backwards into the stem of a plant through the cut end of a petiole. It seemed reasonable to suppose that virus juice might equally well be used in this type of experiment and that the juice would pass directly into the xylem vessels of the main stem. The juice used in these experiments was filtered under pressure through macerated filter paper, or through a filter paper impregnated with fuller's earth, which removed all the solid material present in the macerated tissue. It was found that of this juice 1–4 c.c. were absorbed at the cut end of the petiole in 24 hours. The lamina of a leaf was removed under water and the cut end of the petiolar stump was inserted immediately thereafter into a small tube full of the extracted juice.

A large number of plants have been so treated, about eighty in all. It has been found that in the majority of cases where the petiolar stump

had been left attached to the plant after treatment the plant showed symptoms of disease in the usual manner after a period of incubation. It is not surprising that in these cases all the plants did not take virus disease. To ensure that the vessels would be of large size well-grown plants were taken, for example, about  $1\frac{1}{2}$  feet in height, and at this stage only under very exceptional conditions do more than a small majority of the plants develop symptoms even after inoculation by leaf mutilation. When young plants were used, though the amount of inoculum absorbed was very much less than that absorbed by the older plants, the incidence of infection was much higher, often 100 per cent., which is found also with leaf mutilation. The amount of living tissue exposed at the end of a cut petiole is naturally very small and, as has been seen, the virus agent travels only in the living tissues. When the petiole, following treatment as described, was removed from the plant with a sterilised scalpel after a period of less than 48 hours, it was found that the plant did not develop any symptoms of disease. In every case the petiole was removed close to the stem with a red-hot scalpel—being rather burnt than cut off. As a consequence, all the living tissues near the point of cutting were completely destroyed. This had the effect of preventing any chance infection arising from the liberation of infectious juice from the xylem vessels and the accidental inoculation of the living tissues around. Only four out of the sixty plants so treated ultimately developed symptoms of aucuba mosaic, and these symptoms appeared so long after treatment as to suggest the possibility of secondary, accidental infection. The results of some of the experiments are summarised in the appended table.

Table I.  
*Experiments on petiolar absorption of virus juice.*

Date of experiment	Petiole removed within 48 hours		Petiole left attached	
	No. of plants	No. infected	No. of plants	No. infected
26. v. 30	4	0	4	0
14. vi. 30	—	—	8	7
20. vi. 30	—	—	8	6
14. vii. 30	6	0	6	2
18. vii. 30	3	0	3	2
13. viii. 30	6	0	6	5
Totals	19	0	35	22

It was concluded from these experiments that the virus agent was travelling with the juice in the xylem vessels of the main stem, but that there was no mechanism whereby it could get out of those vessels to infect the living cells of the mesophyll, etc. In the former instances, where the petiole had been left attached to the plant for some time after

treatment, the virus agent had had time to infect the exposed living tissue, to multiply in it and to travel through the protoplasmic connections into the cells of the main stem and thence into the plant as a whole. Apparently, the time taken by the agent to enter the broken cells at the end of the petiole, to infect the living tissue round it and pass into the main stem is considerable. Part of the time must necessarily be taken up in the multiplication of the virus, but a proportion is taken up in the movement of the agent across the living tissues. The rate of this movement is demonstrably slow, and this, along with other facts, suggests that movement takes place along the protoplasmic strands rather than as a mass movement along the phloem elements.

In an attempt to follow the path of the virus juice in the plant after absorption had taken place at the cut end of a petiole methylene blue or eosin was mixed with the juice. The fact that the virus agent passes unaltered through fuller's earth and other negatively charged colloids suggests that it itself carries, or that it is associated with some substance carrying, a negative charge. This consideration, coupled with the fact that the walls of the xylem vessels are slightly negatively charged, suggested the use of eosin in experiments demonstrating the absorption of the virus juice. The main difficulty which arises in experiments with eosin is that the dye is toxic and, if in sufficient concentration to be easily seen in the xylem vessels of the plant, is strong enough to kill the tissues and, ultimately, the whole plant. To avoid this difficulty, methylene blue was used. This dye carries a positive charge and is, therefore, slightly adsorbed by the walls of the vessels. This is no argument against its use in this connection but rather a point in its favour, since, if it can pass over a given region despite adsorption, clearly a substance of comparable size which has not been adsorbed and which, therefore, normally would move more rapidly, should also have travelled. This dye was used in fairly high concentration also and it was found that those plants which absorbed through a cut petiole the methylene blue-virus juice solution did not subsequently develop disease symptoms. It was also found that when a mixture of methylene blue and juice was inoculated into the lamina of a leaf in the usual manner of inoculation by leaf mutilation, the plants did not develop virus symptoms. Symptoms developed however after inoculation with the same methylene blue-juice preparation when diluted and the agent did not appear to be inactivated, when, after treatment with strong methylene blue, the juice was passed through fuller's earth. This led to the detailed examination of the plants, the petioles of which had been treated with strong

methylene blue and juice, when it was found that the living cells at the end of the petiole and near the injected vessels, and also those in the mesophyll round the needle holes in the laminae which had been inoculated, had been killed as a result of the toxic effects of the methylene blue. In these plants, therefore, the probability is that the virus agent had not actually come into contact with the living cell and, therefore, the plants could not truly be said to have been inoculated with the virus agent.

THE EFFECT OF CRUSHING LEAVES AFTER INJECTION OF THE XYLEM  
ELEMENTS WITH VIRUS JUICE.

As has been seen, there is no evidence that the virus agent is able to enter the living cells of a plant if it be moving about in the xylem stream. As it was clear that the juice had entered the xylem vessels and was, presumably, being carried up in the water stream to the leaves, it appeared reasonable to expect that if, in the experiments where no disease symptoms had followed the removal of the treated petiole, the virus agent had been allowed access to the living tissue the plants would have become infected. When this type of experiment was carried out it was found that crushing of the leaves above the treated petiole did give rise, in some cases, to the appearance of symptoms.

The method adopted was as follows. The cut end of a petiole was inserted into filtered juice. After an interval of 24–48 hours the treated petiole was removed from the plant with a hot scalpel. The leaves directly above the treated petiole were crushed with sterilised forceps. Only those leaves were crushed into which it was presumed that the virus juice had passed. The passage of the eosin was taken as a criterion in this case. A solution of eosin in the infective juice was sucked up by a plant, and was used as a control. The appearance of such a plant is shown in Plate XVII. As a consequence of the crushing, the contents of the xylem vessels were brought into contact with the living cells of the mesophyll. In the first series of experiments all the plants treated (six) developed symptoms of disease after the usual inoculation period. In another, nine out of thirteen plants with the leaves crushed became diseased. The success of this method depends on the distribution of the agent in the plant and on its effectual entry into the protoplast of a mesophyll cell. The amount absorbed and, consequently, the amount which is carried along the xylem vessels is, obviously, dependent on the tension which exists in the xylem vessels. Therefore, while on some occasions the movement of juice into the plant was rapid and the amount absorbed considerable, on others the rate of ingress was slow and the

amount absorbed small. Again, there tends to be a precipitation of colloidal and other materials when the filtered juice stands overnight and, when this precipitate is heavy and the rate of inflow low, the xylem vessels at the cut end of the petiole tend to become blocked with detritus of one kind or another. In such cases, the amount of material passing into the xylem stream is small, the concentration of the agent tends to be very considerably reduced and its distribution in the plant rather limited. As a result of these factors, the non-success of some experiments is hardly a matter for surprise. Sometimes none of the plants treated developed symptoms at all. On the other hand, in no experiments did the controls, of which the leaves had not been crushed after the removal of the petiole, develop any symptoms of aucuba disease. Clearly the actual crushing of the leaves of tomato plants has caused the appearance of symptoms only when the virus agent has been present as a consequence of inoculation of some kind. The results of some of the experiments are summarised in Table II.

Table II.

*Experiments on petiolar absorption, with removal of petiole and crushing of leaves.*

Date of experiment	Plants treated	Infection after crushing leaves
28. vii. 30	6	6
13. viii. 30	6	5
15. ix. 30	13	9

#### EXPERIMENTS ON THE ABSORPTION OF VIRUS JUICE BY THE PETIOLES OF PLANTS WITH STEAMED STEMS.

It was thought desirable to combine the technique adopted in the experiments here reported with that adopted in the earlier experiments before discussed (2). The middle portion of the stem of some plants was killed with steam in the manner described. Thereafter, the distal portion of a petiole on the lower part of the plant was removed under water and the end of the stump immediately inserted into filtered virus juice. The plants were then divided into two groups. In the first group, the treated petiole was removed with a red-hot scalpel within 24 hours close to the stem; in the second, the petiole was left attached. The upper leaves of the plants of the first group were crushed with sterilised forceps, and, after the usual period of inoculation, symptoms appeared in the part of the plant above the lesion. The plants of the second group were set aside without further treatment, and, after an appropriate interval, symptoms

appeared on the lower part of the plant. In the first group, on the other hand, the lower part of the plant showed no symptoms. In this experiment, it was therefore clearly shown that it is possible to inject a vascular strand, or strands, with virus juice, and not have infection spreading into the plant as a whole, whereas, if infection takes place as a result of the liberation of the agent from the vessels and its entry into the living mesophyll cells, the agent travels freely about the living tissues of the plant but is unable to move across an area of dead cells and so infect the living cells on the other side.

#### THE ENTRY OF THE VIRUS AGENT INTO THE XYLEM VESSELS.

It is clear that where the virus agent had actually been introduced into the xylem vessels it had moved upward with the water stream into the upper part of the plant, even across the region of dead cells. This does not imply that under normal circumstances the agent could travel in the water stream. It has already been seen that there is apparently no movement of the agent out of the water column and it is, therefore, difficult to see by what mechanism it could normally enter the vessels. With a view to testing this point the following experiment was set up. A series of plants were treated as before described to destroy the living tissue of one of the internodes. The lower part of each plant was then inoculated with aucuba mosaic. The usual method of leaf inoculation was not very satisfactory for this type of experiment, as it is possible that some of the agent might accidentally be inoculated directly into the xylem vessels during leaf mutilation. For this reason, a method of inoculation 'by rubbing the surfaces of the leaves with wool soaked in virus juice was used. In this method the hairs on the surface of the leaf are broken off by friction, and inoculation is made into the exposed protoplasts at the base of each. This method is, probably, more effective as a method of inoculation than most for the reason that it occasions the minimal amount of damage to the tissues (see Holmes(5)). After an interval of 8 days, when the virus had spread freely through the lower part of the plant and symptoms had actually appeared on the leaves of the axillary buds, the upper leaves were crushed in the usual manner. After a further interval of 6 days the upper portion of the plant was removed and was planted as a cutting. In no case did any disease symptoms appear. This experiment has been repeated and in no case has any infection occurred. These experiments, taken in conjunction with the earlier series in which the tissues of the upper portion were found to be non-infectious on inoculation into healthy seedling plants (see Caldwell(2)), lead one to the

conclusion that in the normal plant the virus agent does not travel in the xylem vessels—that, in fact, the virus agent does not enter the xylem vessels, since no mechanism exists which would normally admit of its entry, and that, further, even if the virus agent had entered the xylem vessels and had travelled in the water stream, there exists no evidence that the infectious principle would be able to leave the vessels and to enter the living cells of the mesophyll to set up infection.

THE ABSENCE OF THE VIRUS AGENT FROM THE HYDATHODE EXUDATE  
OF DISEASED PLANTS.

The results which have been outlined and discussed in this paper have all tended to show that the virus agent does not travel in the xylem stream in the normal plants. Only when infectious juice had been introduced directly into the vessels was the agent found to be carried in the water stream. Even under these circumstances, the agent did not cause infection unless the contents of the vessels had been brought into close contact with the living mesophyll cells. For example, it was necessary to crush the leaves in some experiments before any symptoms appeared on the treated plants. It was concluded, therefore, that the agent of aucuba mosaic, at least, was unable to leave the xylem vessels, and it is suggested that a similar difficulty would arise in connection with its entry into the xylem stream. In other words, in the normal diseased plant there is no virus material in the water stream. This point can very easily be tested experimentally. The leaves of the tomato are furnished with hydathodes of a simple type. These function but rarely under ordinary glasshouse conditions. On the other hand, when young plants are put under a bell-jar in a warm glasshouse so that the air inside the bell-jar is rapidly saturated with water vapour, drops of water appear after some time at the tips and along the margins of the leaves. These drops are exuded from hydathodes which are of the type described by Haberlandt as "water stomata" (cf. Haberlandt(3)). In such a hydathode the pore is more or less directly connected with the bundle ends and no secretory process as such is involved in the exudation of water from the pore. Plate XIX, fig. 2, shows the appearance of the pore at the tip of a young tomato leaf. It can be seen from this photomicrograph that it has a similar structure to the water stoma of *Fuchsia* as illustrated by Haberlandt (*loc. cit.*) (cf. Fig. 198 of Haberlandt, *loc. cit.*).

There is, between the bundle end and the pore proper, a group of loosely packed mesophyll cells and no secretory cells such as are characteristic of the glands found on the leaf of *Lathraea* spp. The liquid which

appears is, therefore, approximately of the nature of that found in the xylem stream. It was found that the exudation of water occurred most readily on the younger leaves of the axillary shoots which developed on the stump of a fairly mature tomato plant. The top of a diseased tomato plant was removed, leaving a stump of stem 4–6 inches high with five leaves on it. These leaves were removed and shortly afterwards the axillary shoots appeared. The leaves on these shoots were all badly infected with aucuba mosaic. The plants were put under bell-jars standing in water. After a short time in a warm glasshouse, drops of water appeared at each of the hydathodes. These were collected and inoculated into healthy seedling tomatoes. All the seedlings grew and remained healthy. Three sets of six plants were used in this experiment. As a control, leaves were removed from the same experimental plants and the drops of water which collected at the cut end of the petiole were inoculated into young seedlings. These drops, it was suggested, were contaminated by traces of the contents of the cut cells at the ends of the petiole. All the seedlings inoculated with this water developed symptoms. It was concluded, therefore, that only a trace of virus, as has already been shown by various workers, was required to infect the seedlings, and that even this trace was absent from the water exuded from the hydathodes. The water in the vascular tissue was therefore apparently free from virus.

#### EXPERIMENTS WITH CUTTINGS WHICH HAVE BEEN KEPT IN VIRUS JUICE.

A simple method of demonstrating the non-movement of the virus agent from the xylem stream has been tried. The upper 6–9 inches of the stem of young tomato plants was removed and the end put into filtered virus juice. After 24 hours the tops were divided into three groups. The first group was planted as cuttings directly. The second was planted after the lower 2 inches of stem had been cut off with a red-hot scalpel. The third group was treated as was the second, and after planting some of the leaves were crushed with a pair of sterilised forceps. All the plants of the first group developed symptoms of mosaic as a result of the treatment to which they had been subjected. None of the plants of the second developed symptoms, because, as has been shown, the agent had not had time to pass upwards through the living tissue before the end was removed. Of the third group four of the eight plants treated developed symptoms of mosaic.

A similar set of experiments was carried out to ascertain if the leaves of plants with virus juice in the xylem vessels afforded suitable



material for inoculation. Two groups of cuttings were used. In the first the ends of the cuttings were placed in filtered virus juice for 24 hours. In the second the middle portion of each of the stems was steamed before the ends were placed in similar juice for a like period. The upper leaves of both sets were taken, macerated in the minimum amount of water and inoculations made from them. The plants inoculated developed symptoms of mosaic after the usual period.

The results of these experiments again come into alignment with those of the earlier experiments and confirm the results obtained therefrom.

#### DISCUSSION.

These experiments raise some new points which might be discussed at this stage. It appears that the virus agent can enter only slightly injured cells and may be incapable of passing directly into uninjured ones. As has been seen (4), neither in the case of virus juice placed on unbroken hairs on the surface of a leaf, nor in the case of that present on the inner boundary of living cells, *i.e.* in the xylem stream, do symptoms appear in the plant.

In these experiments, in which concentrated methylene blue was inoculated with the infectious juice, the tissues round the site of inoculation were killed and, as has been noted, the plants did not subsequently show symptoms of disease. This is attributed to the fact that movement apparently does not take place across a region of dead tissue. This explanation accounts also for the fact recorded by Holmes (5), *viz.* that washing off the inoculum from infected *N. tabacum* tissue on leaves of *N. glutinosa* "never decreases the number of successful inoculations, and may increase the number, especially if the fluid sample containing the virus to be measured also contains some substances harmful to the tissues of the inoculated plant." These observations affect the interpretation of results where the precipitants used in the preparation of virus material are themselves toxic to living cells. One might have been inclined to the view, which apparently has no justification from the subsequent experiments, that methylene blue in high concentration had inactivated, perhaps by adsorption, the virus agent. This point is dealt with more fully in a subsequent section.

#### THE NON-MOVEMENT OF THE VIRUS AGENT THROUGH KILLED MESOPHYLL CELLS.

Mention has been made of the difficulty of successfully inoculating plants with virus agents in the presence of toxic substances in the inoculum. It is suggested that, even where the amount of toxin is not

sufficient to kill large portions of the lamina, too severe injury to the cells round the points of inoculation may successfully prevent entry of the virus agent into the general plant body. This point is illustrated by some of the experiments on the effect of precipitates on virus juice. Holmes(5) finds that the rubbing of the lamina to break the hairs is a more effective method of inoculation than leaf mutilation—a point which seems to support the view that badly injured cells do not allow of the multiplication of the virus agent. The rubbing method is by far the most efficient for use with aucuba mosaic—100 per cent. infection taking place even with fairly mature plants.

The whole question of the technique of inoculation seems to be involved in this consideration. Tobacco mosaic and aucuba mosaic of tomato are both so infectious that little difficulty attends their successful inoculation into healthy plants. Even with these, however, there is some evidence to show that the less injury done to the inoculated tissues the greater will be the chances of infection. It is possible, though so far there is no evidence on this point, that in some of those instances where successful insect infection is comparatively easy and successful needle inoculation rare or absent, the non-success of needle inoculation is to be accounted for on the grounds that too much damage is done to the surrounding tissues and that insect attack is more efficient and less destructive. The insects which do act as vectors are, typically, insects with efficient sucking apparatus and not biting insects which crush and destroy the tissues of a leaf.

#### EXPERIMENTS ON THE REMOVAL OF THE AGENT FROM INFECTIVE JUICE.

There seems little doubt that the agent of aucuba mosaic itself carries, or is associated with some substance carrying, a negative charge. In many experiments with fuller's earth, which has been found an excellent material for removing much of the colloid material from plant juices before inoculation, there has been no occasion on which the infectivity of the treated juice has been obviously impaired as a result of treatment. There does not appear to be any saturation of the charges in the fuller's earth by the virus agent, as is found in the case of acid aluminium hydroxide. In this latter case, small quantities of juice after passage through the hydroxide may no longer be infective (cf. Allard(1)). The amount of the reduction of the infectivity seems to be associated with the volume of the juice which passed over the hydroxide, the positive charges thereon being readily saturated so that the negatively charged agent is no longer adsorbed (cf. Rhoads(6)).

With these facts established, it appeared that positively charged dyes, such as methylene blue, would be valuable as indices of the amount of movement of the virus agent in the xylem vessels. The negatively charged colloids of the virus juice should, theoretically, travel more readily than the positively charged methylene blue. As has been seen, when strong methylene blue was used the juice absorbed by the petioles did not, even when the petioles were left on the stem, infect the whole plant with mosaic. This, it is suggested, was due to the toxic effect of strong methylene blue and not to the inactivation of the agent by adsorption (cf. Vinson and Petre(9)).

An attempt was made to discover if the usual protein precipitants removed the virus agent from infectious juice. An acid solution of mercuric sulphate (see West, Scharles and Peterson(10)) was first used. It was found that a few drops of this precipitant were sufficient completely to discharge all the precipitable materials from the juice. When this was done and the precipitate made up with water and inoculated into plants no symptoms appeared. Neither did they appear when the filtrate was used as the inoculum. When, however, in a second set of experiments, the precipitate and the filtrate were neutralised with dilute alkali before inoculation the precipitate was found to contain the virus agent apparently unaltered. The filtrate was non-infectious even after treatment with sodium sulphate and zinc to remove any traces of toxic mercuric salts. In the case of the precipitate, the toxic effect of the acid had been removed and the virus agent was able to enter the living protoplasts of the mesophyll cells round the points of inoculation.

Other precipitation methods were tried. The fact that the material has to be heated in the method involving the use of zinc hydroxide makes this method unsuitable for the testing of the effect of this precipitant on the agent. Fairly prolonged heating on a water bath at 100° C. will inevitably destroy the agent.

The lead acetate method, on the other hand, is suitable for this investigation, and it was found that, when sufficient lead acetate was added to precipitate completely the proteins of the juice of tomato leaves infected with aucuba mosaic, all the virus appeared in the precipitate: the filtrate contained no virus as judged by inoculation into healthy seedlings even after the removal of the excess lead acetate with sodium carbonate.

EXPERIMENTS ON THE MOVEMENT OF THE VIRUS AGENT  
THROUGH LIVING TISSUES.

The experiments recorded above, together with those described in the earlier paper, indicate that movement of the virus agent tends to be, normally, along the protoplasmic strands, entry being first made through a broken protoplast. To test this hypothesis, a series of experiments was set up to determine the rate of movement of the agent both upwards and downwards in the same plant. The rate of movement has been found already for the agents of some tomato virus diseases by Boning and this work has been noted in the earlier paper of this series. On the other hand, some workers (see Holmes (4, 5)) have found that, in other instances, the upper portion of the plant becomes diseased much earlier than the lower, the interval being, in the case of tobacco, one of some weeks. It is rather important in the light of this difference of opinion to establish whether or not movement is approximately at the same rate upwards and downwards in the stem of tomato. If the main movement of the virus were always in one direction (either up or down), then, presumably, there would be a *prima facie* case for the contention that movement was taking place along one of the organised conducting channels. If it be shown that movement tends to be upwards and downwards at apparently the same rates in a single plant, then the presumption is that the movement of the agent is not confined to any of the conducting systems.

The fact that symptoms normally appear at the top of the plant first need not, obviously, be taken as evidence of a greater movement in that direction. The presence of meristematic tissue, it is now generally considered, is necessary for the active multiplication of the virus agent. To avoid the difficulty attendant on this consideration, a series of experiments was set up, in which the axillary buds all along the stem were made available as the centres of active virus multiplication. In a typical experiment of this kind a plant was inoculated on a leaf which was rather more than half-way up the stem. The inoculation was made on the pinnae of the fifth leaf. After 3 days the plant was carefully cut up into sections with due precautions against accidental infection. In each section there was an axillary bud. The pieces of the stem were planted as cuttings. There were six cuttings, each with an axillary bud, the top and the root portions of the stem, the latter having the axillary bud of the first leaf. The leaves were numbered from below upwards. Within 11 days, the leaves of all the cuttings except those with the first and second axillary buds had developed symptoms of the disease. The distance travelled by

the agent in 3 days was, therefore, three internodes upwards as against two downwards—probably a difference so slight as to be ignored, though there is usually slightly greater movement upward than downward. In other experiments it was found that the virus agent had travelled downwards in the tomato plant 40 cm., *i.e.* down 20 cm. of petiole and 20 cm. of stem, in the case of large plants, in less than 6 days, while it had travelled upwards a slightly greater distance in the same time. In the controls of all the experiments, the upper leaves were always the first to show the symptoms, the leaves of the axillary buds only becoming mottled at a much later stage. These results, which confirm the data of Boning, seem to indicate that there is no rapid movement of the virus agent in any one direction in the plant, but rather that the agent moves slowly up and down the stem from the point of insertion of the inoculated leaf. The slight tendency to quicker movement upward may be associated with the increased rate of multiplication of the agent, which is usually found in meristematic tissues.

#### THE DEVELOPMENT AND MOVEMENT OF THE VIRUS AGENT IN THE PLANT IN THE DARK.

The effect of environmental conditions on the appearance of symptoms and on the development of virus disease in plants is considerable. The main difficulty, which inevitably suggests itself in connection with the interpretation of the results of experiments conducted under different environmental conditions, is that attendant on the impossibility of deciding between the specific effect of the conditions on the agent itself and on the host plant. Clearly, if the metabolism of the host plant is materially altered as a result of the altered environment, the effect on the development of symptoms, etc., might be considerable despite the fact that little alteration of the agent had taken place. In the following series of experiments the effect of new environmental conditions appeared to be slight or completely absent. Eighteen plants about 6 inches high were put into a darkened chamber on August 5th. On the 6th, 24 hours afterwards, they were inoculated with aucuba mosaic, each on the pinnae of the third leaf from the base. Six of them (Series A) were put back into the glasshouse, while the other twelve were put back into the darkened chamber. The inoculated leaf of each of the six plants in the light and of each of six of the plants in the dark was removed on August 10th, 96 hours after inoculation. Thereafter the six darkened plants were placed in the light (Series B). Symptoms appeared on all the plants—both those which had been continuously in the light and those which

had been kept for 4 days following inoculation in the dark—on August 13th and 14th. There was apparently little or no difference between the reaction of the control plants and the experimental plants as regards their response to virus disease. The other six plants were taken out of the dark chamber on August 11th—5 days after treatment (Series C). The treated leaves were removed. These plants, 24 hours later, showed signs of a “permanent wilt,” and the older leaves, especially, were practically all dead, despite the fact that there was no suggestion of water shortage at the roots as shown by the absence of subsequent recovery in the light and on watering. The general appearance of the leaves is illustrated in Plate XX, figs. 1 and 2. On first inspection the appearance suggested a severe necrosis—although thorough examination revealed the fact that the condition of the leaves was typical of desiccation rather than of necrosis. Further work on this “permanent wilt” indicates that it is associated with the carbohydrate content of the leaves, either directly or indirectly, and the data which have been obtained will be given in a later paper.

For convenience, the details of this experiment are set out in tabular form in Table III.

Table III.

Series A	In dark 24 hours	Inoculated	In light thereafter
Series B	“    “	“	In dark 4 days—in light thereafter
Series C	“    “	“	In dark 5 days—in light thereafter

All these plants developed symptoms of aucuba mosaic.

The results so far obtained have shown that there is no evidence that darkness delays the spread of the virus agent in the plant, and that, the virus having spread, symptoms which subsequently appear in the light after the same period of inoculation are of the same type as those which appear in the normal plants. Symptoms do not normally appear in the dark on young plants, nor is this a matter for surprise when it is remembered that tissues of a young normal tomato plant collapse in the dark after 7–10 days' treatment. It must further be remembered that the general yellowing of the tissues of plants kept in the dark would preclude the recognition of mosaic symptoms. Actually, even this collapse of the older leaves is not sufficient to prevent completely the formation of symptoms which do appear on the younger leaves which subsequently develop if the plants are not kept in the dark for too long a period of time. In older plants, the tissues of which are sufficiently supplied with reserve material, etiolation takes place to a lesser or greater extent and the wilting is delayed, but, in the discoloured tissues which appear as a

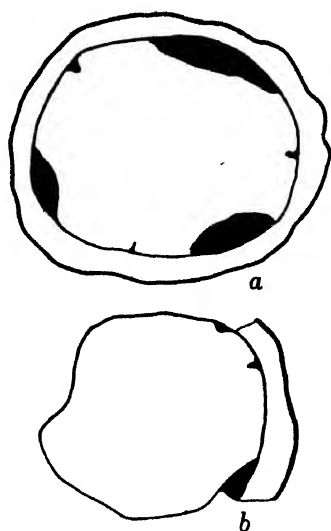
consequence of etiolation, the development of symptoms of aucuba mosaic is very difficult of assessment.

THE UPWARD MOVEMENT OF THE VIRUS AGENT IN STEMS AFTER THE  
REMOVAL OF PARTS OF THE VASCULAR TISSUE.

The evidence presented in this and in the previous paper appears to prove fairly conclusively that the xylem is not the normal path of movement of the agent of aucuba mosaic in tomato, and so far no evidence has been adduced to show that this agent differs radically from those of other virus diseases in this respect. The question of the actual path of movement, however, presents more difficulty. By analogy with the movement of metabolites, and from the experiments on the movement of the agent up and down the plant, it has been suggested that for this virus agent movement seems to be possible in any living cells. That is to say, the agent moves in the phloem rather because the tissue is composed of living cells than because it is transported passively, as are the carbohydrates for example. Some additional evidence which, it is thought, definitely supports this hypothesis, is here presented.

In the stem of the young tomato plant about a foot in height it is possible to see the position of the vascular strands in the internodes. When the plant is held in front of a bright light the strands appear as dark lines against the lighter ground tissue. The removal of portions of the vascular tissue in the internodes presents very little difficulty, therefore, and can be carried out without greatly disturbing the living cells of the cortex and medulla. In a series of plants of this kind portions of the bundles about half an inch long were removed all from one internode so that the continuity of the bundles in the plant was broken. As a consequence, the upper portion of the plants collapsed completely in a very short space of time, and the plants were not suitable for further experimentation. It was noticed, however, that there were in each internode three main bundles and three intermediate small bundles. When the three larger bundles were removed the three smaller appeared to allow of the passage of sufficient water to keep the upper leaves turgid. Similarly when all except one of the larger bundles were removed, that is, when the plant was ringed practically all the way round so as to expose the parenchyma of the pith, sufficient water passed up to keep the tissues above turgid. Various groups of plants were so treated and the damaged tissues heavily smeared with vaseline. The lower leaves were then rubbed with macerated virus material. After intervals of 3 or 4 days the upper portion of each of the plants was removed and planted as a

cutting. For convenience, before treatment the leaves of the middle portion were removed, as illustrated in Plate XVIII. Untreated plants, also with the leaves of the middle portion removed, were similarly inoculated and used as controls. It was found that symptoms appeared on the cuttings of the treated plants after the same interval of time—17–19 days—from the time of inoculation, as on those of the control plants. In another series the tops were not removed after inoculation, and the plants were set aside in the glasshouse to develop symptoms. Symptoms in this case developed on the control plants and on the treated plants after the same interval of time—10 days. In these experiments, again, aucuba mosaic in tomato was used throughout. Text-fig. 1 shows how much of the vascular tissue could readily be removed without substantially impeding the movement of the virus and it is suggested from a consideration of this data that there is strong presumptive evidence that the movement of the agent of aucuba disease of tomato can take place and does take place readily through any living tissue and that the phloem is, in this case, not the main channel of movement in the normal plant.



Text-fig. 1. Outline drawings (projection on same scale) of sections of tomato stems: (a) normal stem; (b) after removal of most of the vascular tissue (cf. Plate XVIII).

#### SUMMARY.

In this paper the results of some experiments with aucuba mosaic in tomato are discussed. These results support the general thesis that the agent does not normally travel in the xylem stream. The movement of tobacco mosaic in tobacco and in tomato was found to be similar to that of aucuba mosaic. The majority of the experiments were carried out with aucuba mosaic in tomato.

It was found that filtered virus juice from virus-infected plants was readily absorbed at the cut end of a petiole and thence travelled into the xylem of the main stem. The removal of the treated petiole within 48 hours prevented infection taking place. On the other hand, when the petiole was left attached the experimental plant developed symptoms in the usual manner. This type of experiment was repeated with the



exception that, after the removal of the treated petiole, the leaves above were crushed. Infection of the plant followed this treatment. This experiment was combined with the earlier experiments in which the living tissue of an internode was killed by steam. The agent was found to be carried mechanically in the xylem across the dead tissue.

As a consequence of this observation the experiments with plants with "steamed" internodes were repeated. It was found that in no case did crushing of the leaves induce symptoms on the upper part of the plant when inoculation had been made on the lower side of the "steamed" internode.

It was concluded that the virus agent did not normally enter the water stream, and when it was introduced experimentally into it, though it was carried round, there was no mechanism by which it could leave the vessels.

The absence of the agent from the hydathode exude was demonstrated.

Apparently the agent cannot enter an unbroken cell, nor can it move through or out of dead cells. It has been found that great care must be taken to ensure the absence of traces of toxic substances from inocula to be tested, otherwise infection may not take place even in the presence of the agent itself.

The rates of movement of the virus agent in the tomato are practically the same upward or downward. The slightly greater rate of upward movement appears to be associated with the greater metabolic activity which occurs in the upper portion of the plant. The movement of the virus agent along the protoplasmic strands has been examined by inoculating plants with infective juice after the removal of large portions of the vascular tissue. This treatment does not appear to delay the movement of the agent up the stem.

In a final group of experiments, darkness did not appear to have any effect on the multiplication of the virus in the tissues. Too prolonged periods in the dark, however, caused the permanent wilting of both diseased and healthy plants. This "wilting" is considered as being due to the respiration of carbohydrates, etc., and the earlier collapse of the diseased plants as being due to a smaller carbohydrate supply in them.

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## EXPLANATION OF PLATES XVII-XX.

## PLATE XVII.

Tomato plant with "steamed" internode, after the absorption of eosin by a petiole on the lower part of the plant.

## PLATE XVIII.

- (a) A control plant, with a few leaves removed and inoculated with aucuba mosaic below;
- (b) plant with vascular tissue of one internode removed (at x). Both plants developed symptoms of aucuba mosaic in the upper parts after the same period. In (b) note the development of the axillary shoots in the lower part of the plant.

## PLATE XIX.

- Fig. 1. The leaves of a young tomato plant with drops of water exuded from the hydathodes.
- Fig. 2. Photomicrograph of the tip of a young tomato leaf showing bundle end and simple type of water stoma.
- Fig. 3. Mass of adventitious roots developed above the "steamed" portion of a tomato stem.

## PLATE XX.

- Fig. 1. (a) Diseased plants (aucuba mosaic); (b) healthy plants. Both sets photographed after 5 days in dark chamber.
- Fig. 2. "Healthy" plants: (a) after fortnight in dark, with well-watered soil; (b) control, kept in light, with dry soil.

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Fig. 1.

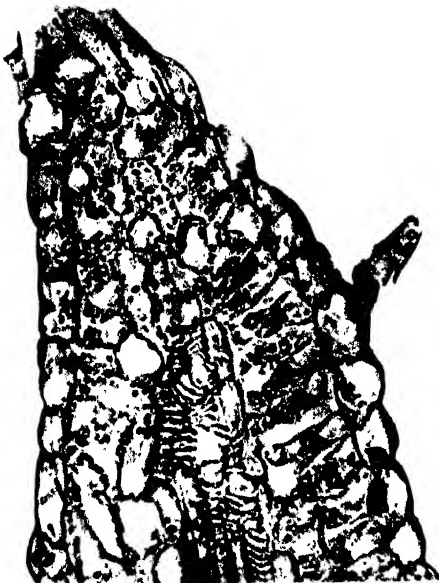


Fig. 2.



Fig. 3.







Fig. 1.



Fig. 2.

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